ABSTRACT

Title of Dissertation: COUPLING ANAEROBIC DIGESTION TECHNOLOGY AND FORAGE RADISH COVER CROPPING TO OPTIMIZE METHANE PRODUCTION OF DAIRY MANURE-BASED DIGESTION

Ashley Juanika Belle, Doctor of Philosophy, 2015

Directed by: Professor Stephanie Lansing
Department of Environmental Science and Technology

Anaerobic digestion technology was coupled with a new forage radish cover cropping system in order to increase biogas production of a dairy manure digester. Specifically, this research investigated forage radish as a renewable source of energy in terms of methane (CH_4) production, the effect of radish co-digestion on hydrogen sulfide (H_2S) production, and the relationship between H_2S production and methanogenesis limitations. Optimal substrate co-digestion ratios and inoculum to substrate ratios (ISR) were determined in the laboratory with biochemical methane potential assays (300 mL) and pilot-scale complete mix batch digesters (850 L) were constructed and operated to determine energy production potential at the farm-scale level.

Laboratory results showed that forage radish had 1.5-fold higher CH_4 potential than dairy manure on a volatile solids basis, with increasing the radish content of the co-digestion mixture significantly increasing CH_4 production. Initial H_2S production also increased as the radish content increased, but the sulfur-containing compounds were rapidly utilized, resulting in all treatments having similar H_2S concentrations (0.10-0.14%) and higher CH_4 content in the biogas (48-70% CH_4) over time. The 100% radish digester had the highest specific CH_4 yield (372 ± 12 L CH_4/kg VS). The co-digestion mixture containing 40% radish had a lower specific CH_4 yield (345 ± 2 L CH_4/kg VS), but also showed significantly less H_2S production at start-up and high quality biogas (58% CH_4). Utilizing 40% radish as substrate, decreasing the ISR below 50% (wet weight) resulted in unstable digestion conditions with decreased CH_4 production and an accumulation of butyric and valeric acids.

Pilot-scale experiments revealed that radish co-digestion increased CH_4 production by 39% and lowered the H_2S concentration in the biogas (0.20%) beyond that of manure-only digestion (0.34% – 0.40%), although cumulative H_2S production in the radish + manure digesters was higher than manure-only. Extrapolated to a farm-scale (200 cows) continuous mixed digester, co-digesting with a 13% radish mixture could generate 3150 m^3 CH_4/month, providing a farmer additional revenue up to $3125/month in electricity sales. These results suggest that dairy farmers could utilize forage radish, a substrate that does not compete with food production, to increase CH_4 production of manure digesters.

COUPLING ANAEROBIC DIGESTION TECHNOLOGY AND FORAGE RADISH COVER CROPPING TO OPTIMIZE METHANE PRODUCTION OF DAIRY MANURE-BASED DIGESTION

By

Ashley Juanika Belle

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Advisory Committee:
Professor Stephanie Lansing, Chair
Professor Richard E. Erdman
Professor Gary Kent Felton
Professor Raymond R. Weil
Dr. Walter Mulbry

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Foreword

Chapter 4 of this dissertation was previously published in *Bioresource Technology's* Special Issue on Thermo-chemical Conversion of Biomass. The published material is research that was conducted and primarily written by Ashley J. Belle during her doctoral studies at the University of Maryland. Ashley is listed as the first author of this publication, with members of her dissertation committee as co-authors. The dissertation examining committee, along with the Department of Environmental Science and Technology's Graduate Director, accept the inclusion of this previously published work in the dissertation as Ashley has made substantial contributions to the work.

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Dedication

This work is dedicated to my parents, Earl and Olivia Belle. Thank you for instilling in me the value of hard work. I love you dearly.

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I am forever grateful to have such a loving and supportive family. To my parents, Earl and Olivia Belle, my brother Jason C. Belle, my aunt Oria Lee Houston, and cousin Angela R. Houston, I especially thank you from the bottom of my heart

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Chapter 1: Introduction

1.1 Background and Rationale for Conducting the Research

Since the pre-industrial era, methane (CH_4) emissions have increased globally by 150% (IPCC, 2013). In the United States, livestock manure management is responsible for 26% of the anthropogenic CH_4 emissions related to agricultural activities, with dairy cattle listed as the highest emitter of all domestic animals (EPA, 2014a). Methane emissions associated with the dairy cattle industry have grown largely due to facilities shifting from solid to liquid manure storage systems in order to abide by nutrient management regulations that limit the application of solids to agricultural fields. The liquid handling of dairy manure creates anaerobic conditions, which lead to the production of CH_4 (EPA, 2014a).

In order to reduce CH_4 emissions associated with the handling of liquid dairy manure, AgSTAR, an outreach program jointly sponsored by the U.S. Department of Agriculture, the U.S. Environmental Protection Agency, and the U.S. Department of Energy, promotes the use of anaerobic digestion (AD) technology as a mechanism of capturing the methane-enriched biogas generated from animal manures and utilizing it as an on-farm renewable fuel source. The biogas can be used to generate electricity, sold as a renewable natural gas source (Naja et al., 2011), or burned for heating and cooking, thus displacing the need for wood and/or charcoal (Lansing et al., 2008; Rahman et al., 2014). Burning of methane-enriched biogas releases carbon dioxide into the atmosphere, which has 1/28 of the greenhouse gas impact that CH_4 has (IPCC, 2013), thus minimizing CH_4 emissions.

Other benefits of AD technology include the reduction of pathogens (Cote et al., 2006) and the production of a liquid fertilizer in which the nutrients are more biologically available to the crops, as organic nitrogen and phosphorus are converted to ammonium and orthophosphate during the digestion process (Maranon et al., 2011). Studies have also shown that AD reduces odor emissions associated with the handling of manures on farms between 70-95%, allowing for homes and farms to coexist (Masse et al., 2011).

Currently, approximately 244 anaerobic digesters are operating in the United States at commercial livestock facilities, with the majority of these digesters at dairy facilities (AgSTAR, 2014a). Worldwide, there are over 20,000 large scale anaerobic digesters, with China and Germany as leading countries, largely due to government credits and subsidy programs (Burns, 2009; Abbasi et al., 2012). In August 2014, the Obama Administration released the *Biogas Opportunities Roadmap*, a voluntary strategy designed to address climate change by reducing CH_4 emissions in the agricultural sector by utilizing CH_4 recovery systems such as anaerobic digesters. With the limited use of AD technology in the U.S., the roadmap seeks to identify ways to expand the use of the technology at U.S. livestock facilities as well as decreasing barriers that all too often thwart installation and successful operation. It was estimated that at full potential, the American biogas industry could produce enough energy to power 1 million homes, providing a win-win situation for farmers who would not only provide power to their own farms but also generate revenue by providing the supplemental energy to neighboring communities (EPA, 2014b).

There are several common barriers associated with the adoption of AD technology in the United States. The lack of financial assistance and high capital costs associated with the installation of current U.S. digestion systems limits the number of sites where such technology would be economically feasible (AgSTAR, 2014b). Many U.S. dairy facilities lack the herd size (≥500 cows) AgSTAR estimates for AD to be economically viable due to being limited by their animal waste capacity (AgSTAR, 2011). In comparison to other digestion feedstocks, manure has relatively low biodegradability and biogas potential (Ward et al., 2008; Mata-Alvarez et al., 2014). Additionally, in order for current U.S. digestion systems to input generated electricity into U.S. infrastructure, connection to power grids with controls is required. The costs, maintenance, and logistics associated with updating infrastructure can outweigh the returns that would be generated from selling electricity due to the low price of electricity in the United States (AgSTAR, 2014b).

Energy production investment returns reveal that dairy digesters are not always economically favorable when dairy manure is digested solely (Klavon et al., 2013). However, biogas production from dairy digesters can become more economically viable by adding additional biodegradable feedstock that is located within close proximity to the dairy facility (El-Mashad et al., 2010). Co-digesting manure with other substrates, such as agricultural waste (Abouelenien et al., 2014), fats, oil, and grease (Lansing et al., 2010), or energy crops (Yue et al., 2013) with higher biogas potential, have been shown to increase CH_4 production up to 3.5-fold higher (Mata-Alvarez et al., 2014), thus increasing the feasibility of AD technology for the small to mid-sized dairy farmer. The Minnesota Project, a non-profit organization promoting sustainable energy production geared towards smaller dairy operations, reported that the addition of cheese whey to dairy manure digesters operating at Jer-Lindy Dairy (Brooten, MN) increased biogas production by 300% (Minnesota Project, 2010). Lansing et al. (2010) utilized plug flow digesters for co-digestion of swine manure and used cooking grease, resulting in a 124% increase in CH_4 production with a 2.5% to 10% addition of grease (by volume). However, the percentage of CH_4 in the biogas decreased with increasing grease additions, likely due to a build-up of volatile fatty acids (VFA), highlighting the importance of obtaining an optimal substrate co-digestion ratio in order to promote CH_4 generation.

Renewable energy production can be achieved by anaerobically digesting a variety of energy crops; however the most widely used energy crops consists of maize (195-402 L CH_4/kg VS) (Gao et al., 2012; Golkowska and Greger, 2013), switchgrass (191-309 L CH_4/kg VS) (Barbanti et al., 2014; Masse et al., 2010), sugar beets (236-381 L CH_4/kg VS) (Umetsu et al., 2006; Braun et al., 2009), sunflower grass (154-400 L CH_4/kg VS), and Sudan grass (213-303 L CH_4/kg VS) (Amon et al., 2007; Braun et al., 2009). In order to obtain the maximum biogas yield from an energy crop, several key factors should be taken into consideration, including selecting the appropriate energy crop (i.e. variety; genotype), harvest time, pretreatment of the biomass prior to the digestion process, and nutrient/mineral composition of the energy crop (Amon et al., 2007). Since plant chemical composition varies over time as the plant matures, harvest time can have an effect on the CH_4 yield of the crop. Thus, in order to maximize the CH_4 yield, the ideal harvest time of the crop for AD should be determined. To date, inconsistent results have been found regarding the effect of harvest time on anaerobically digested energy crops (Lehtomaki et al., 2008), highlighting the need for further research on the effect of harvest time on digestion efficiency.

Popular winter cover crops grown in Maryland include winter rye, winter wheat, oats, forage radish, hairy vetch, and crimson clover. Similar to forage radish, oats will also winter-kill, while winter rye and hairy vetch are hardy plants that will continue growth in the spring (Traunfeld, 2009). Review of literature reveals that winter wheat and winter rye have been studied extensively as AD substrates, with CH_4 potentials ranging from 228-360 L CH_4/kg VS (Amon et al., 2007; Rincon et al., 2010) and 140-360 L CH_4/kg VS (Amon et al., 2007; Petersson et al., 2007), respectively. However, there is limited information on utilizing forage radish as a renewable energy source. The CH_4 production potential of radish can greatly vary based on multiple factors such as plant variety, portion of plant digested, and soil characteristics. Carvalho et al. (2011) determined that oilseed radish (*Raphanus sativus* var. *oleifera* cv. Pegletta) was a good substrate for CH_4 production (300 L CH_4/kgVS). However, it should be noted that oilseed radish (pH 7.64) and forage radish (*Raphanus sativus* var. *longipinnatus*) (pH 4.3-5.5) are of different plant varieties and pH value, thus CH_4 production levels may greatly differ. Furthermore, to our knowledge, no other studies have experimentally determined how the total sulfur content of radish contributes to hydrogen sulfide (H_2S) production in the biogas, the influence of H_2S production on CH_4 production during radish digestion, or the effects of utilizing the radish as a co-substrate in dairy digesters to enhance biogas production. Thus, this research determined the optimal substrate co-digestion (radish and manure) ratio and inoculum level for enhanced methanogenesis while minimizing the H_2S content of the biogas.

Forage radish was selected as the co-digestion substrate to investigate since it is a readily available cover crop that has seen increased interest by corn silage-based dairy farmers in the Northeast U.S. due to its multiple soil and environmental benefits. Typically, dairy farms in the NE region leave the land fallow after harvesting corn silage in August, therefore, the planting of forage radish immediately after corn harvest can be conducted without interfering with food production. Ideally, the winter cover crop is planted from August to mid-September. This crop is attractive to farmers because it increases crop yield (Gruver et al., 2014), does not compete with other food crops (Weil et al., 2009), suppresses early spring weeds (Lawley et al., 2012), and is known as being a bio-driller. The radish roots can penetrate 6 feet or more down into the soil profile thus alleviating soil compaction (Chen and Weil, 2010 and 2011). The crop also reduces nitrogen leaching into the groundwater as the deep penetrating roots are able to capture the nitrogen and bring it to the surface thus increasing topsoil fertility. Additionally, as the crop rapidly decays, it also releases nitrogen early into the topsoil, thus reducing the need for fertilizer (Weil and Kremen, 2007; Weil et al., 2009). Typically, this crop would not be harvested by the farmer, as the crop winter kills and rapidly decays in late December, leaving behind a clean enhanced seedbed (Weil et al., 2009). However, in this research, the above-ground biomass of the radish cover crop was harvested before winter-kill for digestion substrate. Harvesting could expand the use of the radish cover crop and previous research has shown that even when the crop is harvested, the soil benefits previously discussed are still realized (Lawley et al., 2012). Use of the radish as a co-substrate in a dairy digester has the potential to enhance biogas production during the fall and winter months when the demand for fuel is particularly high in the United States.

1.2 Research Objectives and Hypotheses

The overall goal of this research was to couple AD technology with forage radish cover cropping to increase biogas production of dairy manure-based digestion. Specifically, this research investigated forage radish as a renewable source of energy in terms of CH_4 production, the effect of radish co-digestion on H_2S production, and the relationship between H_2S production and methanogenesis limitations.

The main objectives of this research include the following:

Objective 1: To determine the optimal co-digestion ratio of dairy manure and forage radish based on CH_4 production (biogas quantity and % CH_4 in biogas) and H_2S concentration and determine if a synergistic effect on CH_4 production is observed when co-digesting manure and radish cover crops.

Hypotheses: Increasing the ratio of forage radish in dairy digesters will increase CH_4 production until a certain threshold when H_2S production begins to inhibit methanogenic activity. Co-digestion of forage radish in dairy digesters will result in higher CH_4 production than the sum of each substrate digested individually, with forage radish having a higher biogas potential than dairy manure, and the combination having the highest CH_4 production.

Objective 2: To determine the effect of forage radish harvest date (early harvest in October vs. late harvest in December) on CH_4 production.

Hypothesis: The CH_4 potential of the late-harvest radish crop will result in 50% more CH_4 production than the early-harvest crop.

Objective 3: To determine the effect of the inoculum to substrate ratio (ISR) on CH_4 production during forage radish digestion and co-digestion with dairy manure.

Hypothesis: An ISR of 1:1 (by wet weight) will have the greatest production of CH_4 in the generated biogas, with significant decreases in CH_4 production at lower ISRs and no statistically significant changes at higher ISRs.

Objective 4: To determine the effect of VFA production and pH on CH_4 production at varying ISRs.

Hypothesis: Decreasing the ISR will result in an unstable digester with decreases in pH value and CH_4 production and an accumulation of VFAs.

Objective 5: To determine the difference in CH_4 and H_2S production when digesting only dairy manure versus co-digesting dairy manure with forage radish cover crops in batch pilot-scale complete mix digesters.

Hypothesis: Co-digesting with radish will significantly increase CH_4 and H_2S production relative to manure-only digestion.

Objective 6: To determine how the percentage of forage radish in the co-digestion mixture affects CH_4 production in batch pilot-scale complete mix digesters.

Hypothesis: Increasing the radish content in the co-digestion mixture will increase CH_4 production.

Objective 7: To quantify the radish crop acreage required for co-digestion at the farm-scale level and how inclusion of radish cover crops affects on-farm energy production potential.

1.3 General Research Approach

Forage radish cover crops (above-ground biomass) and dairy manure were used as digestion substrates. Several acres of forage radish were grown each year of this project at a USDA-ARS facility located in Beltsville, MD. The methane potential of the substrates was determined at two scales. To achieve Objectives 1-4, optimal substrate co-digestion ratios and inoculum to substrate ratios were determined in the laboratory with biochemical methane potential experiments (300 mL). For laboratory experiments, the radish was harvested by hand from randomized 1 m^2 quadrants. For Objectives 5-7, six pilot-scale batch complete mix digesters (850 L) were designed, constructed, and operated during two 33-day field trials. For the pilot-scale experiments, a rotary mower and forage chopper were used for radish harvesting and to determine biomass yields. Digestion efficiency and stability were evaluated by analyzing biogas samples for CH_4 and H_2S and liquid samples for pH, total and volatile solids, soluble chemical oxygen demand, volatile fatty acids, total Kjeldahl nitrogen, total Kjeldahl phosphorus, and total sulfur.

Chapter 2: Methane and Hydrogen Sulfide Production during Co-digestion of Forage Radish and Dairy Manure

2.1 Abstract

Forage radish, a winter cover crop, was investigated as a co-substrate to increase biogas production from dairy manure-based anaerobic digestion. Lab-scale batch digesters (300 mL) were operated under mesophilic conditions (35°C) during two experiments (BMP1; BMP2). In BMP1, the optimal co-digestion ratio for radish and dairy manure based on CH_4 production and H_2S concentration was determined by increasing the radish content (above-ground biomass) from 0–100% (wet weight). Results showed that forage radish had 1.5-fold higher CH_4 potential than dairy manure on a volatile solids basis. While no synergistic effect on CH_4 production resulted from co-digestion, increasing the radish content of the co-digestion mixture significantly increased CH_4 production. Initial H_2S production increased as the radish content increased, but the sulfur-containing compounds were rapidly utilized, resulting in all treatments having similar H_2S concentrations (0.10-0.14%) and higher CH_4 content in the biogas (48-70% CH_4) over time. The 100% radish digester had the highest specific CH_4 yield (372 ± 12 L CH_4/kg VS). The co-digestion mixture containing 40% radish had a lower specific CH_4 yield (345 ± 2 L CH_4/kg VS) but also showed significantly less H_2S production at start-up and high quality biogas (58% CH_4). Results from BMP2 showed that the radish harvest date (October versus December) did not significantly influence radish C:N ratios or CH_4 production during co-digestion with dairy manure. These results suggest that dairy farmers could utilize forage radish, a readily available substrate that does not compete with food supply, to increase CH_4 production of manure digesters in the fall/winter.

2.2 Introduction

The economics of dairy manure-based digesters are not always favorable due to the relatively low biodegradability and biogas yield of dairy manure compared to other organic wastes (El-Mashad and Zhang, 2010; Klavon et al., 2013; Wang et al., 2011). To increase methane (CH_4) production from manure-based digesters, appropriate co-digestion substrates can be used. Determining the appropriate substrate ratios is a key factor in creating an optimized anaerobic digestion (AD) environment, as the composition of each substrate can vary greatly in characteristics such as alkalinity, pH, organic content, nutrient composition, and microbial population (Ward et al., 2008; Pages-Diaz et al., 2014; Al Seadi et al., 2008). Umetsu et al. (2006) showed that increasing the percentage of sugar beet tops (10% - 40%, by volume) in dairy manure digesters increased CH_4 production up to 45%. However, while increasing the proportion of sugar beet roots from 5% - 12% increased CH_4 production by 11%, inhibitory effects were observed for mixtures containing 15% beet roots. Zhang et al. (2013) showed that the CH_4 yield decreased as the ratio of food waste to cattle manure increased from 2:1 – 4:1 (VS basis), whereas Bah et al. (2014) demonstrated that as the ratio of palm pressed fiber to cattle manure increased from 1:3 to 3:1 (VS basis), CH_4 yield significantly increased.

Co-digestion has also been shown to have synergistic and antagonistic effects on CH_4 production. A synergistic effect results in increased CH_4 production from the substrates beyond what is achieved from digestion of each individual component, while an antagonistic effect negatively influences CH_4 production likely due to inhibitory substances or toxicants present in the mixture components (Pages-Diaz et al., 2014; Navaneethan et al., 2011). A synergistic effect was observed when co-digesting equal fractions (wet weight (ww) basis) of municipal solid waste, slaughterhouse waste, manure, and various crops. Due to the mixture satisfying more of the nutritional demands of the microbial community, co-digestion increased CH_4 production by 31% compared to the CH_4 production value of each individual fraction (Pages-Diaz et al., 2014). Li et al (2013) observed that co-digesting corn stover and chicken manure at a 3:1 ratio (VS basis) had a greater synergistic effect (14% increase) on CH_4 production compared to a 1:1 ratio (4% increase). Antagonistic effects were observed co-digesting calcium acetate with boiler cleaning waste, with large reductions in CH_4 production as the proportion of boiler cleaning waste increased, likely due to inhibitory concentrations of copper and chromium present in the cleaning wastewater (Navaneethan et al., 2011).

The chemical composition of plant tissue commonly varies over time as the plant matures, which can potentially affect the CH_4 yield when utilizing plant material as co-digestion substrate. Lehtomaki et al. (2008) found no significant change in CH_4 yield when a vetch-oat mixture was digested after harvest at the vegetative or flowering stages. However, the same study showed that while reed canary grass and giant knotweed produced the most CH_4 if harvested during the late flowering stage, rhubarb and lupine produced the most CH_4 if harvested during the vegetative stage. Masse et al. (2010) determined that the CH_4 yield of switchgrass decreased with advancing stages of development, and Ragaglini et al. (2014) found that the juvenile crop stages of giant reed had the highest CH_4 production. In contrast, Bruni et al. (2010) found that fresh maize at late harvest had the highest CH_4 yield. These inconsistent results highlight the need for further research on the effect of harvest time on digestion efficiency and led Lehtomaki et al. (2008) to conclude that the effect of harvest time on AD was crop specific.

This study investigated coupling AD technology with a new forage radish cover cropping system in order to increase biogas production during the fall and winter months. With over 80% of the U.S. agricultural AD systems operating on commercial dairies (AgSTAR, 2014), this co-digestion research was designed to assist small to mid-sized corn-silage based dairy farmers in overcoming limitations of low biogas production from dairy manure by providing a readily available co-digestion substrate that does not compete with food production. Radish cover crops have not been studied extensively as a potential bioenergy feedstock for AD. To our knowledge, there are only a few studies that have utilized radish crops in mono-digestion studies, while co-digestion studies are limited to one report utilizing radish fodder (whole plant) and pig slurry at one substrate mixture ratio (75% pig slurry & 25% radish, by ww) (Peu et al., 2013). Review of previous mono-digestion studies revealed that the CH_4 potential of radish varied greatly (237-450 L CH_4/kg VS) depending on plant variety, soil characteristics, climatic conditions, the portion of the plant digested, and pretreatment methods (Gunaseelan, 2004; Peu et al., 2012; Carvalho et al., 2011; Nielsen and Feilberg, 2012; Molinuevo-Salces et al., 2013).

If the proportions of co-digestion mixtures are arbitrarily selected, the full CH_4 potential from the combination of substrates may not be realized (Pages-Diaz et al., 2014). The aim of this research was to determine the optimal substrate ratio for co-digesting the above-ground biomass of fresh forage radish cover crops and dairy manure (0 to 100%, by ww). Utilizing only the above-ground radish biomass allows for easier harvesting of the cover crops using typical farming machinery, such as a mower and forage chopper, and the nitrogen taken up in the roots will remain in the field and become available for the subsequent corn crop as the below-ground root decays (Weil and Kremen, 2007; Weil et al., 2009). The transformation of sulfur-containing compounds in the radish to hydrogen sulfide (H_2S) in the produced biogas was also determined across the entire range of co-digestion ratios as forage radish is a sulfur-rich crop and H_2S can corrode biogas utilization systems. The specific research objectives were to: (1) determine the optimal co-digestion ratio of dairy manure and forage radish above-ground biomass based on CH_4 production and H_2S concentration, (2) determine whether inclusion of forage radish to dairy manure has a synergistic effect on CH_4 production, and (3) determine the effect of forage radish harvest date on CH_4 production. Utilizing forage radish, a cover crop that would otherwise winter-kill, could potentially enable dairy farmers to produce additional renewable energy without losing the benefits of the cover crop. Even if the above-ground radish biomass is harvested for AD, the benefits of increased topsoil fertility, compaction alleviation, and weed suppression will remain (Chen and Weil, 2010; Chen and Weil, 2011; Lawley et al., 2012).

2.3 Materials and Methods

2.3.1 Feedstocks

Forage radish cover crops (*Raphanus sativus* var. *longipinnatus*) and dairy manure (both scraped manure and the liquid fraction of solids-separated manure) were used as digestion substrates. The above-ground biomass of the forage radish cover crop was harvested from a USDA facility located in Beltsville, MD (39.03°, -76.89°). Planting of the radish cover crop occurred in August immediately after corn silage harvest. The radish was harvested by hand prior to winter-kill from randomized 1 m^2 quadrants. A stainless steel knife was used to harvest the above-ground biomass, which consisted of the leafy shoots plus a small portion of the fleshy root that extended above the soil surface. Each radish was cut approximately 3 to 5 cm from the soil surface and frozen in heavy-duty plastic bags until use. After thawing, a food processor was used to chop the radish into a semi-slurry material in order to emulate harvesting the radish utilizing a rotary mower and forage chopper.

Dairy manure was obtained from the 120-cow USDA research dairy facility. The dairy manure was scraped and stored in a manure pit prior to solids separation with a FAN separator, which removes roughly 80% of the solids. The liquid fraction of the separated manure is treated in a mesophilic (25-35°C) complete-mix anaerobic digester (540 m^3). Inoculum from this digester was obtained from a sampling port located inside the digester and was utilized to accelerate biogas production in the batch studies.

2.3.2 BMP1: Optimal co-digestion ratio experimental design

Biochemical methane potential (BMP) assays were conducted to determine the CH_4 potential of each substrate individually and the optimal co-digestion ratio. The BMP assay determines the relative biodegradability of an organic material by a consortium of anaerobic microbes under batch conditions. BMP1 assays were based on a modified method of Moody et al. (2011) using 21 glass serum bottles (300 mL), with three replications of seven treatment groups: manure only (0% radish), radish only (100% radish), and co-digestion mixtures containing radish and dairy manure (the liquid fraction of solids-separated manure) with 20, 40, 50, 60, and 80% radish addition. All substrates were added on a ww basis (Table 2.1). BMP1 was conducted for 30-days, the time period in which biogas production had largely ceased, with daily biogas production contributing < 1% of the cumulative biogas production.

An equal quantity of inoculum was added to each BMP bottle, resulting in the inoculum to substrate ratios (ISR) ranging from 3.3:1 – 1.5:1 by g VS, which was similar to Raposo et al. (2006) where the ISR ranged from 3:1 – 1:1 by g VS and showed little variability in the CH_4 yield coefficient. Three additional BMP bottles containing inoculum-only served as seed blanks. The inoculum had an average pH of 7.53, and total solids (TS) and volatile solids (VS) concentration of 19.4 and 11.9 mg/g, respectively. Nutrient media was not utilized as dairy manure has been shown to contain the necessary micronutrients for digestion (Al Seadi et al., 2008).

The bottles were purged with N_2:CO_2 gas (70:30 vv) to displace residual oxygen and capped with a rubber septum to create an anaerobic environment. The bottles were placed on a continuous orbital shaker (New Brunswick Scientific; Edison, NJ USA; model Innova 2300) at 117 RPM and incubated in a darkened environmental chamber at 35^{o}C.

2.3.3 BMP2: Effect of harvest date experimental design

In order to determine the effect of the harvest date on CH_4 production potential of forage radish cover crops, an additional BMP experiment (BMP2) was conducted. BMP2 was conducted for 60-days in batch mode with three replications of two treatment groups: 23% early-harvested radish and 23% late-harvested radish (by ww) as co-substrate with scraped dairy manure (77%, by ww). The early-harvested radish was collected on October 22 and had an average TS and VS concentration of 98.5 and 89.3 mg/g, respectively, while the late-harvested radish was obtained prior to winter-kill on

December 15 and had an average TS and VS concentration of 103 and 90.7 mg/g, respectively. The VS concentrations of the radish substrates were found to not be significantly different (p-value= 0.112). All treatments contained an equal amount of manure (38.7 and 28.7 mg/g TS and VS, respectively) and inoculum (14.7 and 9.30 mg/g TS and VS, respectively) (Table 2.2). Three additional BMP bottles containing inoculum only served as seed blanks. The bottles were anaerobically incubated as described in Section 2.2.

2.3.4 Biogas analysis

The biogas production and CH_4 and H_2S content of the produced biogas were measured daily for the first week, every other day during the second week of the study, bi-weekly, and weekly, with the frequency of biogas measurement based on the quantity of biogas produced. The produced biogas was quantified by volumetric displacement using a wetted glass gas-tight graduated syringe (50 mL) (Popper & Sons, Inc.; New York USA) inserted through the rubber septum of the BMP bottle into the gas headspace with pressure displacing the syringe plunger until equilibrium. The biogas was analyzed for CH_4 and H_2S content using a gas chromatograph (Agilent Technologies, Inc.; Shanghai China; model 7890 A) with a thermal conductivity detector at 250°C, an HP-Plot Q capillary column (Agilent J&W; USA), and He as the carrier gas at 8.6 ml/min. The oven operated at 60°C for 2 min and subsequently ramped at 30°C/min to 240°C. To account for biogas production from residual biodegradable material in the digester inoculum, triplicate controls containing only inoculum were incubated and sampled simultaneously to allow subtraction of biogas production not attributed to the substrates.

Table 2.1 Feedstock loading for BMP1: Optimal co-digestion ratio study.

	Inoculum *(g_{ww})*	**Radish** *(g_{ww})*	**Manure** *(g_{ww})*	**Total** *(g_{ww})*	**Inoculum** *(g VS)*	**Radish** *(g VS)*	**Manure** *(g VS)*	**Total** *(g VS)*	**ISR**[a]
0% Radish	100	0	10	110	1.19	0.00	0.36	1.55	3.32
20% Radish	100	2	8	110	1.19	0.16	0.29	1.64	2.68
40% Radish	100	4	6	110	1.19	0.32	0.22	1.72	2.24
50% Radish	100	5	5	110	1.19	0.39	0.18	1.77	2.08
60% Radish	100	6	4	110	1.19	0.47	0.14	1.81	1.93
80% Radish	100	8	2	110	1.19	0.63	0.07	1.90	1.69
100% Radish	100	10	0	110	1.19	0.79	0.00	1.98	1.51

[a] Inoculum to substrate ratio (ISR) calculated on a VS basis.

Table 2.2 Feedstock loading for BMP2: Effect of harvest date study.

	Inoculum *(g_{ww})*	**Radish** *(g_{ww})*	**Manure** *(g_{ww})*	**Total** *(g_{ww})*	**Inoculum** *(g VS)*	**Radish** *(g VS)*	**Manure** *(g VS)*	**Total** *(g VS)*	**ISR**[a]
Early Harvest Radish	125	6	20	151	1.16	0.535	0.57	2.27	1.05
Late Harvest Radish	125	6	20	151	1.16	0.544	0.57	2.28	1.04

[a] Inoculum to substrate ratio (ISR) calculated on a VS basis.

2.3.5 Feedstock characterization

The forage radish and dairy manure for BMP1 were characterized for crude protein, acid detergent fiber, neutral detergent fiber, lignin, crude fat, sugar, starch, and sulfur (Cumberland Valley Analytical Services, Hagerstown, MD) (Table 2.3). Co-digestion mixtures were analyzed before and after digestion at the University of Maryland for pH, TS, and VS. The pH was determined with a glass electrode and an Accumet AB 15 pH meter. Standard Methods for the Examination of Water and Wastewater (APHA, 2005) were used to determine TS (Method 2540B) and VS (Method 2540 E). In addition for BMP2, the carbon to nitrogen (C:N) ratio of the radish shoots and roots were determined by combustion utilizing a CHN Analyzer (LECO Corporation; St. Joseph, Michigan; CHN-2000).

Table 2.3 Substrate characteristics for BMP1.

Property	**Dairy Manure**	**Forage Radish**
pH	8.07 (0.01)	5.62 (0.01)
TS *(mg/g)*	47.5 (0.08)	91.6 (1.56)
VS *(mg/g)*	35.9 (0.13)	79.0 (1.46)
Ash *(mg/g)*	11.6 (0.05)	12.7 (0.10)
Crude Protein *(mg/g)*	7.78	12.7
Acid Detergent Fiber *(mg/g)*	16.0	15.3
Neutral Detergent Fiber *(mg/g)*	24.1	18.5
Lignin *(mg/g)*	4.37	1.74
Crude Fat *(mg/g)*	1.23	3.02
Sugar *(mg/g)*	0.85	31.3
Starch *(mg/g)*	3.42	1.28
Sulfur *(mg/g)*	0.14	0.55

Mechanical harvesting of the above-ground radish biomass transforms the radish into a semi-slurry material. The semi-slurry material was dewatered with a French press and the liquid was captured, resulting in 98% recovery of the radish biomass for VS analyses. The VS content was determined for the following fractions of harvested radish (above-ground biomass): semi-slurry, dewatered, and liquid.

2.3.6 Statistical analysis

The experimental design for BMP1 was a completely randomized design with 21 experimental units (BMP bottles) and seven treatment levels (percentage of radish in co-digestion mixture) with three replicates of each treatment. Significance of differences were determined using a single factor ANOVA followed by Tukey-Kramer's post hoc tests for average CH_4 yields, H_2S yields, and VS concentrations using *SAS 9.3* (SAS, Cary, NC). Simple linear regressions were also conducted with percent radish in the co-digestion mixture being the explanatory variable. CH_4 yields, H_2S yields, TS and VS reductions were the response variables studied. For BMP2, two treatment levels (harvest date) with three replicates each were utilized with significant differences in CH_4 yields, C:N ratios, TS, and VS determined by t-tests. The level of significance was held at 0.05 for all statistical analyses. Reported values are given as means with standard errors.

2.4 Results and Discussion

2.4.1 BMP1: Optimal co-digestion ratio

2.4.1.1 Methane production

Methane production increased as the radish content increased from 0% to 100% (Figure 2.1), revealing that co-digestion with radish does increase CH_4 production from dairy manure-based digesters. Utilizing 0% radish (manure-only) and 100% radish as substrates individually, produced 8.7 ± 0.2 and 29.4 ± 0.9 L CH_4/kg substrate, respectively. Co-digesting the same amounts of radish and manure from the individual treatments produced 19.6 ± 1.4 L CH_4/kg substrate, which was only 2.7% different than the combined total from individual digestion (19.0 L CH_4/kg substrate), revealing no synergistic effect when co-digesting the substrates.

Radish content and CH_4 production had a linear relationship ($R^2 = 0.97$) equating to CH_4 production (L CH_4/kg substrate) equaling 0.20x + 9.51, with “x” being percent radish in the co-digestion mixture (Figure 2.2). Even with a small quantity of radish added to the co-digestion mixture (20%), CH_4 production increased by 61% compared to manure-only digestion (p-value = 0.001). The CH_4 production of the 40% radish mixture was significantly greater than 20% radish and 60% radish was greater than 40% radish (Figure 2.1; Tables 2.4, C.1). Increasing the radish content from 60 to 80% radish resulted in CH_4 production values that were not significantly different (p-value = 0.261). However, it should be noted that 80% radish (24.3 ± 1.3 L CH_4/kg substrate) had the most variability among the treatments. Based on the linear regression equation, the 80% radish mixture should have had a higher average CH_4 production value (25.5 L CH_4/kg substrate) than what was experimentally determined.

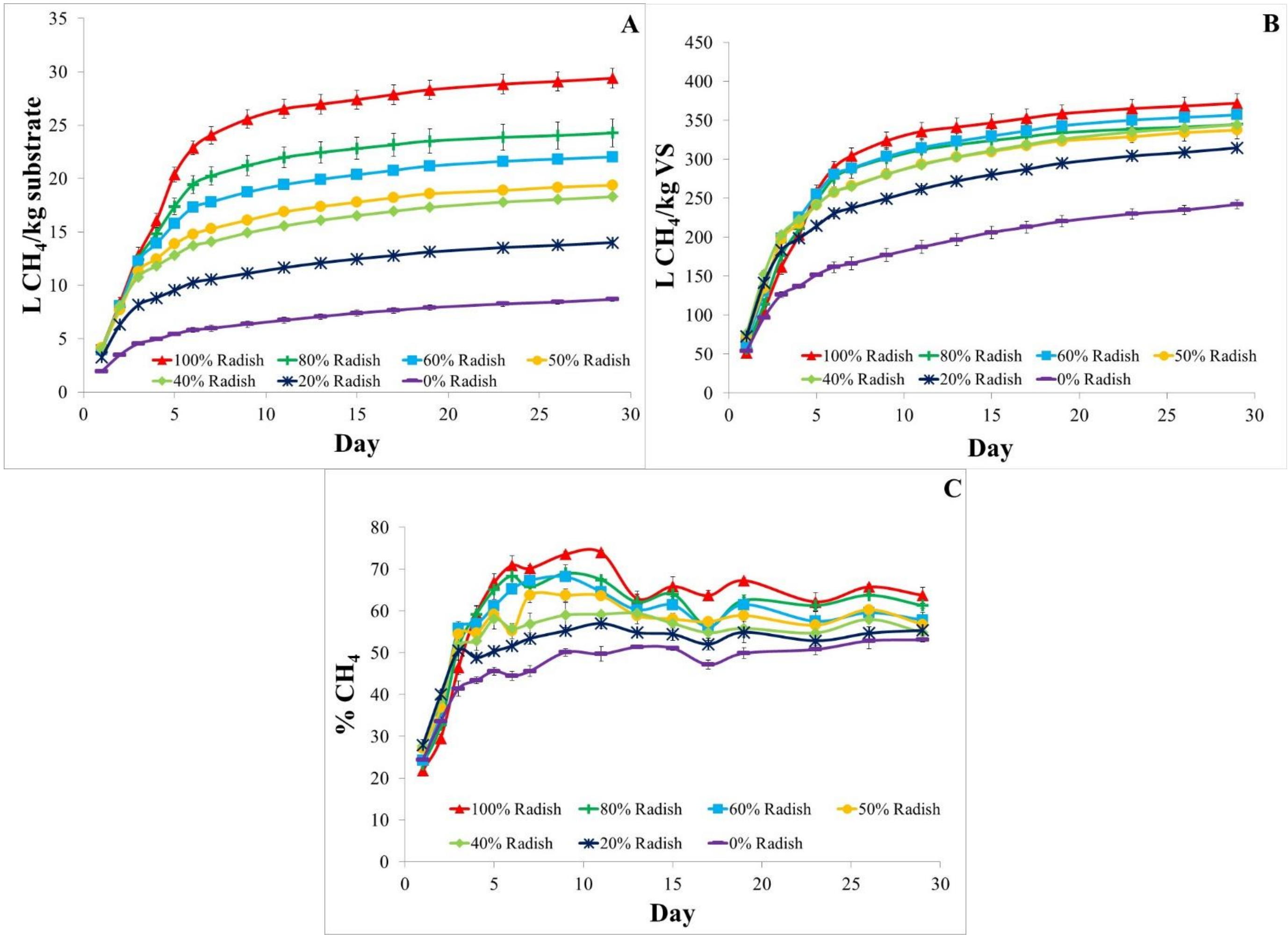

Figure 2.1 Average (± standard error) cumulative CH_4 production during digestion of forage radish and dairy manure mixtures. **(A)** L CH_4/kg substrate added, **(B)** L CH_4/kg VS of substrate added, and **(C)** CH_4 content in the biogas.

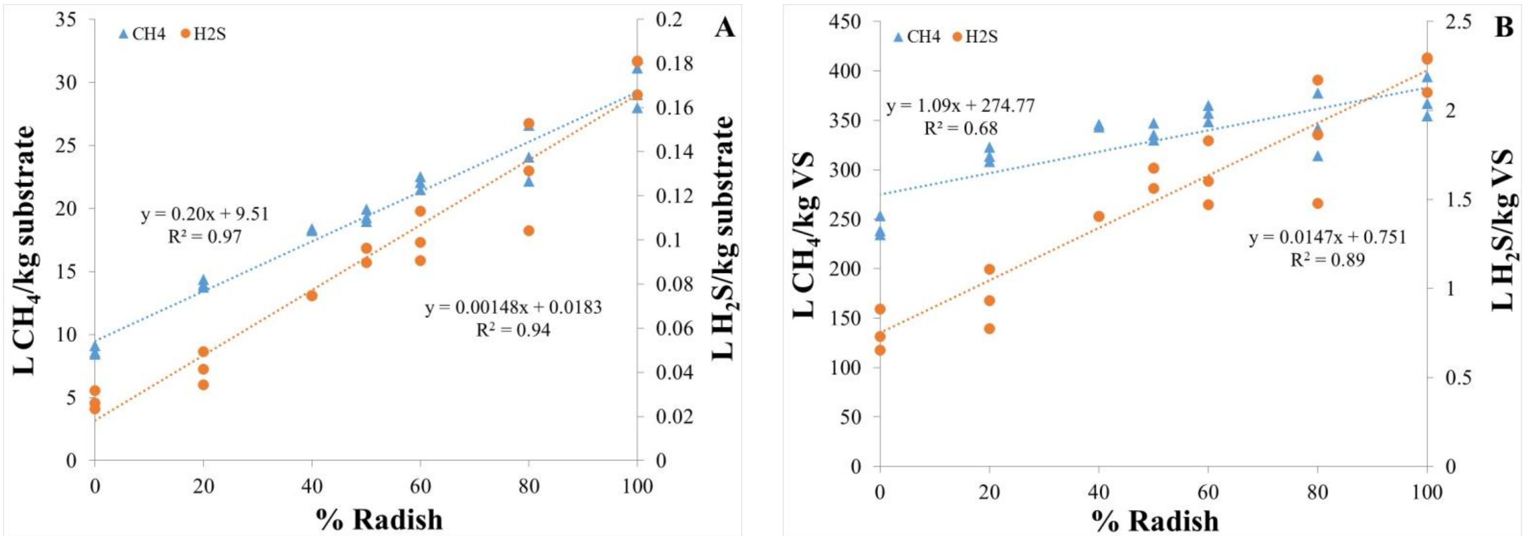

Figure 2.2 Relationship between radish content and CH_4 and H_2S production, respectively. Data normalized by **(A)** kilograms of substrate added and **(B)** kilograms of volatile solids of substrate added.

Table 2.4 Average (± standard error) cumulative CH_4 and H_2S production for BMP1.*

	L CH_4/kg substrate	L H_2S/kg substrate	L CH_4/kg VS	L H_2S/kg VS
0% Radish	8.69 (0.21)^{a}	0.03 (0.002)^{a}	242 (6)^{a}	0.76 (0.07)^{a}
20% Radish	14.0 (0.2)^{b}	0.04 (0.004)^{ab}	315 (4)^{b}	0.94 (0.10)^{ab}
40% Radish	18.3 (0.1)^{c}	0.07 (0.0001)^{bc}	345 (2)^{bc}	1.41 (0.001)^{bc}
50% Radish	19.4 (0.3)^{cd}	0.09 (0.002)^{c}	337 (5)^{bc}	1.64 (0.04)^{c}
60% Radish	22.0 (0.3)^{de}	0.10 (0.01)^{cd}	357 (5)^{bc}	1.63 (0.10)^{c}
80% Radish	24.3 (1.3)^{e}	0.13 (0.01)^{d}	345 (18)^{bc}	1.84 (0.20)^{cd}
100% Radish	29.4 (0.9)^{f}	0.18 (0.01)^{e}	372 (12)^{c}	2.23 (0.06)^{d}

* Superscripts denote significant differences within column.

Normalized by VS addition (specific methane yield), 100% radish had 1.5-fold higher CH_4 potential than 100% dairy manure (Figure 2.1). The addition of radish (20%) to dairy manure significantly improved the conversion of VS into CH_4 by 30% (73 L CH_4/kg VS) (p-value = 0.002). The 100% radish mixture had the highest CH_4 conversion efficiency (372 L CH_4/kg VS), with no significant differences between the specific methane yields of 20-80% radish. However, the conversion efficiency of 100% radish was significantly greater than 20% radish. The similar conversion efficiencies with the inclusion of the radish substrate illustrates that the additional VS from the radish was able to be converted into CH_4.

Hidalgo and Martin-Marroquin (2014) demonstrated that on a VS basis the most CH_4 production also occurred when digesting waste vegetable oil (WVO) and pig manure at a ratio of 1:0, followed by 3:1, 1:1, and 1:3. Dias et al. (2014) also showed that with co-digestion of pear waste with liquid dairy cattle manure that CH_4 production increased from 112.5 to 472.0 L CH_4/kg VS as the percentage of pear waste increased (0 to 100%, by volume) in the mixture. They did note that as pear waste increased, the pH of the digesters decreased (8.60 - 6.44) illustrating acidification could lead to digester instability. However in the radish digestion studies, pH values remained relatively stable, even at higher loadings of forage radish, likely due to the large amount of inoculum utilized to ensure adequate buffering capacity.

2.4.1.2 Hydrogen sulfide production

On a VS basis, 100% forage radish produced significantly more cumulative H_2S than dairy manure (2.23 ± 0.06 and 0.76 ± 0.07 L H_2S/kg VS, respectively) (p-value = < 0.0001) (Figure 2.3; Tables 2.4, C.2). While the addition of a small quantity of radish

(20%) significantly increased CH_4 production, there was no significant increase in H_2S production compared to manure-only digestion (p-value = 0.725). A positive linear relationship (R^2 = 0.94) was observed between radish content and H_2S production equating to H_2S production (L H_2S/kg substrate) equaling 0.00148x + 0.0183, with "x" being percent radish in the co-digestion mixture (Figure 2.2). Increasing radish content in 20% intervals from 20 to 40% radish or 40 to 60% radish, there were no significant differences in H_2S production (p-values = 0.093 and 0.250, respectively). However, 60% radish had significantly higher H_2S production than 20% radish (p-value = 0.001), with 100% radish having the highest H_2S production (0.18 ± 0.01 L H_2S/kg substrate). While the co-digestion mixtures containing a larger proportion of radish (80 and 60% radish) had significantly higher H_2S production values (L/kg substrate) initially, by Day 6, the H_2S concentration (0.10 – 0.14%) of all treatments was not significantly different.

On Day 1, digesters containing a larger radish proportion (≥ 60% radish) had a lower quality of biogas (22 – 25% CH_4) and H_2S concentrations in the biogas being greater than 0.26%. The 100% radish digester had the highest H_2S concentration at 0.37% on Day 1, while the manure-only digester had only 0.17% H_2S (Figure 2.3). As the sulfur-containing compounds in the radish were rapidly utilized and reduced to H_2S, large volumes of H_2S was produced initially. However by Day 6, H_2S production decreased and the CH_4 concentration of the biogas increased, with the CH_4 concentration in all radish digesters being greater than 50%, surpassing the CH_4 concentration of the manure-only digester (46%) (Figure 2.1). As the total sulfur concentration increased with increasing radish addition, the metabolic activity of the sulfate reducing bacteria (SRB) likely also increased, resulting in greater H_2S production initially with the digesters containing a larger radish proportion. As the sulfur was rapidly utilized, the metabolic activity of the SRB declined, resulting in all digesters having similar biogas H_2S concentrations by Day 6. Allen et al. (2014) demonstrated that increasing the content of fresh *Ulva lactuca,* a sulfur-rich green seaweed, from 25 to 75% inclusion with dairy manure (VS basis) decreased the percentage of CH_4 in the biogas from 51 to 25%, with the 25% *Ulva* co-digestion mixture having the lowest H_2S concentration. A large inhibitory effect on CH_4 production with increased radish addition was not found in our study considering cumulative CH_4 production values and CH_4 concentration during peak production.

Overall, methanogenesis was not suppressed by the sulfur content of the forage radish as large amounts of CH_4 were produced (315 – 372 L CH_4/kg VS) relative to manure-only digestion (242 L CH_4/kg VS). After the initial period of CH_4 suppression, as radish content increased the quality of the biogas increased illustrating that co-digesting also improved overall gas quality. At peak CH_4 production (Day 5-15), the CH_4 concentration of the radish digesters ranged from 53 – 70%, with 100% radish having the highest CH_4 concentration. The H_2S concentration ranged from 0.10 – 0.14% for all digesters. These levels exceeded the recommended H_2S concentration for boilers (< 0.10%) (Persson and Wellinger, 2006) and combined heat and power units (< 0.01-0.05%) (Deublein and Steinhauser, 2011) during peak CH_4 production. However the H_2S concentrations of the radish digesters were not significantly different (p-value= 0.056) than the manure-only digester during peak CH_4 production, thus suggesting that no further biogas treatment measures will be required as a result of radish inclusion beyond that which is needed for manure-only digestion.

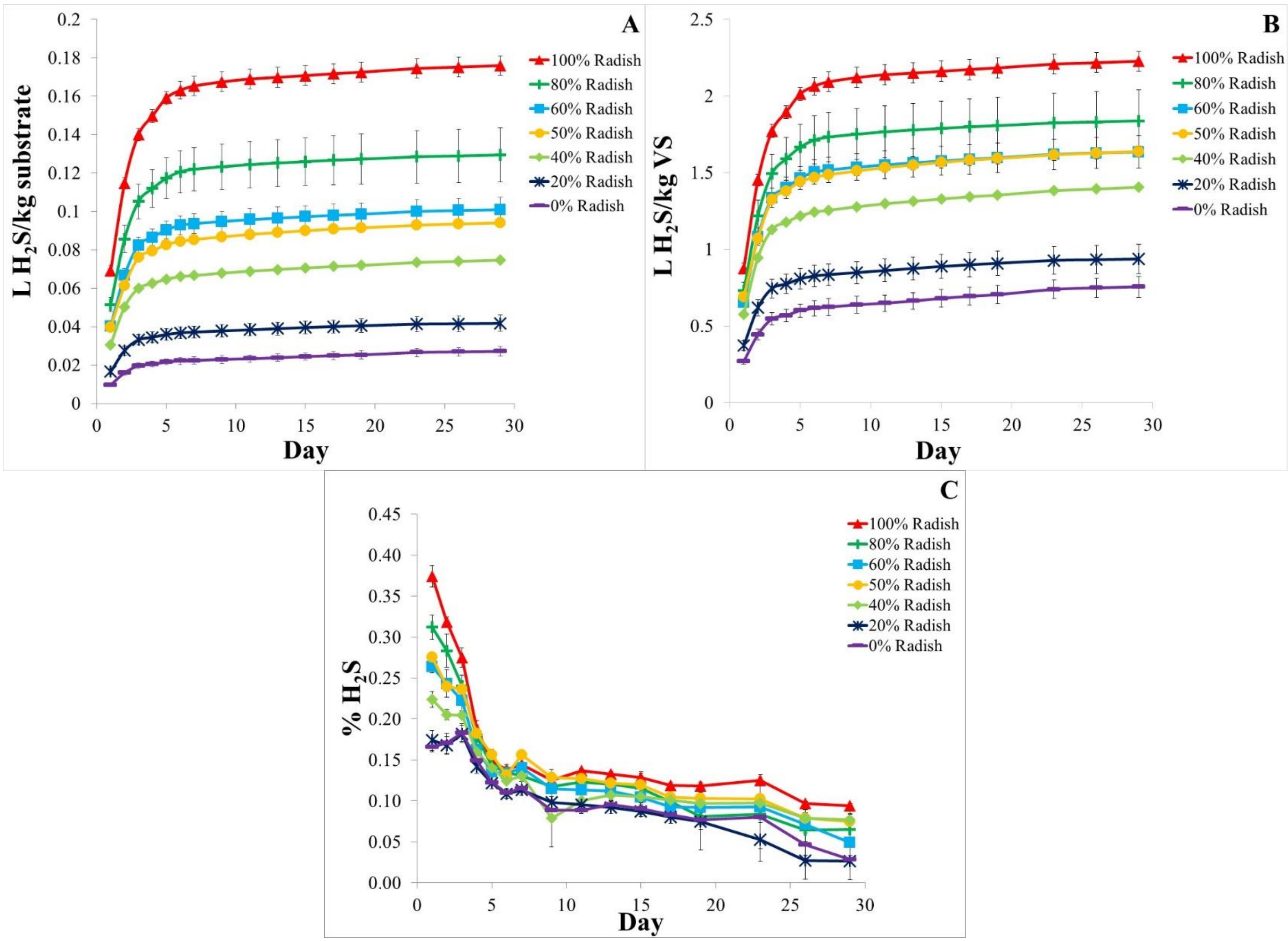

Figure 2.3 Average (± standard error) cumulative H_2S production during digestion of forage radish and dairy manure mixtures. **(A)** L H_2S/kg substrate added, **(B)** L H_2S/kg VS of substrate added, and **(C)** H_2S content in the biogas.

Based solely on highest CH_4 production, 100% radish was the optimal digestion ratio. The VS to CH_4 conversion efficiency was similar with 40-100% radish. A typical field-scale digester (i.e. plug-flow; complete-mixed) may not be designed to accommodate the high solids content of the radish (9.2% TS) nor may a dairy farm digester be available solely for winter-time digestion of radish cover crops. Thus, having a co-digestion mixture containing a smaller proportion of radish may be more conducive to digester maintenance and operation. Since no differences in CH_4 production efficiency (VS basis) were observed from 40 to 100% radish nor between 20 and 40% radish, with 40% radish producing more CH_4 than 20% radish, in real-world operating conditions, it is likely that 40% radish would be a more feasible co-digestion ratio. Utilizing a 40% radish co-digestion mixture would optimize CH_4 production of a dairy manure-based digester, yield high quality biogas (58% CH_4), and have significantly lower H_2S production at start-up. Considering a large amount of inoculum (ISR 10:1, by ww) was utilized in this experiment for radish digestion, future work will consist of determining the minimal amount of inoculum required. While inoculum provides alkalinity and the necessary microorganisms to accelerate biogas production, the biogas potential of inoculum is low because the material has been previously digested. Thus, it is desirable to reduce the inoculum level, particularly in a batch-loaded digester, in order to save space for substrate that has higher biogas potential.

2.4.1.3 pH, organic matter transformations, and radish fractionation

While the radish substrate had a pH of 5.6, the addition of inoculum and/or manure provided a well buffered system in which no pH adjustments were required. The pH of all treatments before and after digestion were within the optimal range for mesophilic AD (6.5 to 8.0) (Al Seadi et al., 2008). Although radish addition to dairy manure was shown to significantly increase CH_4 production, the total VS reductions observed were low (24-40%) (Table 2.5), as this included the inoculum VS as well. This could be attributed to utilizing such a small amount of substrate in comparison to the largely recalcitrant inoculum.

Forage radish was found to have higher biodegradability than dairy manure. While the efficiency in converting VS to CH_4 were similar as radish content increased in the co-digestion mixture, the VS reductions increased as radish content increased (R^2 = 0.95) (Figure 2.4) likely due to the treatments containing larger radish additions had more VS initially and the VS of the radish was more readily degradable than the manure. However, the final VS concentrations for all treatments were similar (10.3 – 11.5 mg/g).

The VS results of the fractionated radish showed that dewatered radish had the highest VS content (130 ± 0.5 mg/g) followed by the semi-slurry material (75.7 ± 2.5 mg/g) and the liquid component, which was significantly lower at 39.5 ± 0.1 mg/g (p-value = < 0.0001). As expected, dewatering the radish concentrated the VS, but also required additional energy to remove water from the radish substrate and reduced the quantity of readily available dissolved VS in the substrate. The semi-slurry material, as harvested from the field, contained 58% of the total VS concentration of the dewatered radish. The CH_4 production potential of the dewatered and liquid radish fractions would reveal if pretreatment was beneficial. The energy required for dewatering could potentially negate any energy gains from digestion.

Table 2.5 Characteristics of BMP1 before and after digestion, showing averages (± standard error).

	pH		**TS** *(mg/g)*			**VS** *(mg/g)*		
	Initial	*Final*	*Initial**	*Final*	*TS Reduction, %*	*Initial**	*Final*	*VS Reduction, %*
0% Radish	7.55 (0.01)	7.41 (0.03)	22.0	18.8 (0.03)	14.2	14.1	11.1 (0.03)	21.3
20% Radish	7.50 (0.01)	7.35 (0.003)	22.8	18.7 (0.2)	17.9	14.9	11.3 (0.1)	24.1
40% Radish	7.52 (0.01)	7.35 (0.004)	23.6	19.3 (0.1)	18.3	15.7	11.5 (0.1)	26.7
50% Radish	7.56 (0.01)	7.36 (0.003)	24.0	18.8 (0.1)	21.4	16.1	11.1 (0.1)	30.8
60% Radish	7.50 (0.02)	7.36 (0.003)	24.4	18.0 (0.1)	26.1	16.5	10.4 (0.1)	36.5
80% Radish	7.48 (0.02)	7.38 (0.01)	25.2	17.9 (0.04)	28.7	17.2	10.4 (0.1)	39.7
100% Radish	7.44 (0.01)	7.39 (0.01)	26.0	18.0 (0.1)	30.6	18.0	10.3 (0.1)	42.6

*Initial TS and VS values were calculated from the individual feedstock values and therefore no standard error values are given.

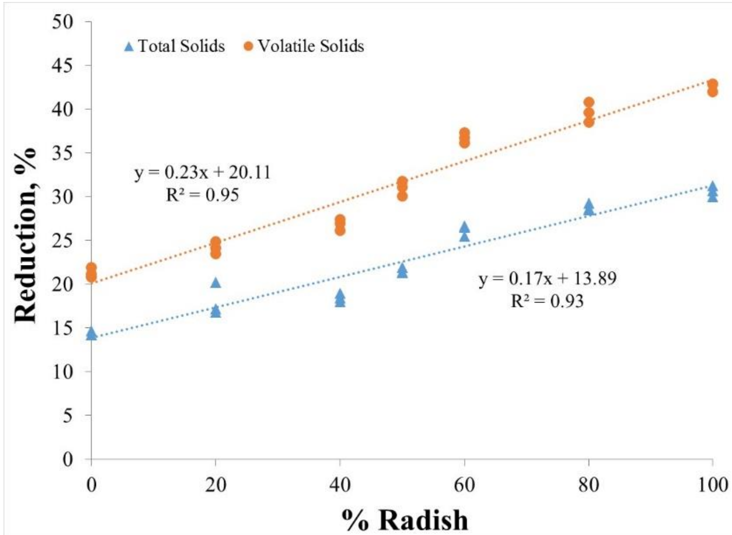

Figure 2.4 Relationship between radish content and TS and VS reduction, respectively.

2.4.2 BMP2: Effect of harvest date

C:N ratios were determined for the radish shoots and roots separately throughout the growing season. As a result of fertilizer being injected into every other corn row in June and inefficient nitrogen uptake by the corn crop preceding the radish cover crop planting, bands of residual fertilizer remained in the soil approximately 150 cm apart. The forage radish growing directly over the residual fertilizer bands (high N zone) was distinctly larger and greener than the forage radish growing over the un-fertilized corn rows (low N zone). As the crop matured, the C:N ratio generally increased (Figure 2.5). There was a greater increase in the C:N ratio of the roots during the growing season compared to the shoots. The above-ground radish biomass, consisting of primarily shoots from the high and low nitrogen zones, were harvested, homogenized, and utilized as the co-digestion substrate. The C:N ratios of the above-ground radish substrates did not change significantly over time (p-value = 0.09), with the early-harvested shoots C:N ratio being 13.3 ± 0.9 and the late-harvested shoots being 15.3 ± 0.5. Although the C:N ratio of the roots (24 – 28:1) was found to be closer to the optimal AD C:N ratio (25-30:1) (Ward et al., 2008), the above-ground biomass was selected for ease of harvesting and to retain nutrient benefits from the root upon decay into the top soil.

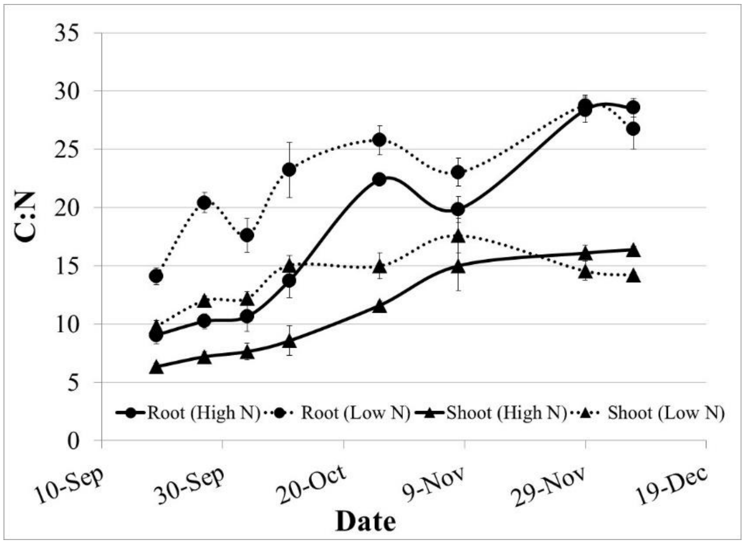

Figure 2.5 Carbon to nitrogen (C:N) ratios for the forage radish cover crops roots and shoots. High N and Low N correspond to the level of nitrogen amendment present in the field during growth of the radish.

The BMP results revealed that forage radish harvest date did not affect CH_4 production during co-digestion with dairy manure. Co-digestion experiments utilizing early harvest (October) and late harvest (December) radish substrate yielded cumulative (during 60 days) CH_4 production values of 15.1 ± 0.01 L CH_4/kg substrate and 14.9 ± 0.3 L CH_4/kg substrate, respectively (Table C.3), with approximately 66% CH_4 in the biogas. Since harvest date was shown not to influence CH_4 production, dairy farmers have the opportunity to incorporate forage radish into their dairy digesters as early as October when the ambient air temperature begins to decline and maintain enhanced CH_4 production throughout the crop's growing season.

2.5 Conclusions

Forage radish cover crops are a suitable co-substrate for optimizing CH_4 production in dairy manure digesters. Increasing the percentage of radish in the co-digestion mixture increased CH_4 production and digestion efficiency in converting VS into CH_4 was similar for the co-digestion mixtures. As radish content increased, initial H_2S production significantly increased. However, H_2S production rapidly declined over time allowing for the CH_4 content of the biogas to greatly increase compared to manure-only. No synergistic effect on CH_4 production was observed during co-digestion. Harvest date of the cover crop did not influence CH_4 production suggesting that the radish can be harvested and added to a dairy manure-based digester from October until winter-kill to increase CH_4 production. Co-digestion with radish cover crops has the potential to increase the economic viability of AD technology, as forage radish was found to have 1.5 times more CH_4 potential than dairy manure on a VS basis.

2.6 Acknowledgements

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Chapter 3: The Effect of Inoculum to Substrate Ratio on Forage Radish Cover Crop Digestion and Co-digestion with Dairy Manure

3.1 Abstract

Biochemical methane potential experiments were conducted to determine the effect of inoculum to substrate ratio (ISR) on CH_4 production. Two substrates, 100% forage radish and a co-digestion mixture containing 40% radish (by wet weight) and dairy manure, were tested. Results revealed that the ISR and substrate composition strongly influenced CH_4 production, with higher ISRs and higher radish content increasing CH_4 production. At an ISR containing 65% inoculum, co-digestion of radish with manure yielded high CH_4 production (284 ± 0.4 L CH_4/kg VS), while the 100% radish produced only 6 ± 0.9 L CH_4/kg VS. However, increasing the ISR to 91% inoculum, specific CH_4 yields significantly increased to 345 ± 2 L CH_4/kg VS and 372 ± 12 L CH_4/kg VS for 40% and 100% radish, respectively. Regardless of the substrate type utilized, digestion instability was observed at ISRs containing ≤ 35% inoculum, resulting in an accumulation of butyric and valeric acids, low pH values (≤ 5.64), and negligible CH_4 production.

3.2 Introduction

Researchers are increasingly co-digesting with energy crops and agricultural residues to increase the economic viability and biogas production from manure-based anaerobic digestion (AD) (Abouelenien et al., 2014; Mata-Alvarez et al., 2014; Wei et al., 2014; Yue et al., 2013). Energy crops, such as maize (195 – 402 L CH_4/kg VS) (Gao et al., 2012; Golkowska and Greger, 2013), sorghum (260 – 390 L CH_4/kg VS) (Chynoweth et al., 2001), and switchgrass (191 – 309 L CH_4/kg VS) (Barbanti et al., 2014; Masse et al., 2010), and agricultural residues, such as fruit and vegetable wastes (290 – 472 L CH_4/kg VS) (Dias et al., 2014; Zuo et al., 2014), have been shown to have higher methane (CH_4) production potential than dairy manure (150 – 190 L CH_4/kg VS) (Belle et al., 2015). However, the introduction of co-substrates into a manure-based digester could potentially disrupt digestion stability if the system does not have adequate buffering capacity to maintain the circumneutral pH (6.5 – 8.0) necessary for methanogenic microorganisms. During mesophilic digestion (30 – 42^{o}C), pH values below 6.0 or above 8.3 can be inhibitory (Al Seadi et al., 2008).

Anaerobic digestion is a microbial-based process, which involves four major stages: hydrolysis, acidogenesis, acetogenesis, and methanogenesis. In hydrolysis, complex organic matter such as carbohydrates, proteins, and lipids are degraded by hydrolytic bacteria into simple soluble organic molecules. Through acidogenesis, volatile fatty acids (VFA) are formed from simple soluble organic molecules by acidogenic bacteria. In acetogenesis, VFAs and alcohols are converted by acetogens into acetate, hydrogen, and carbon dioxide, which are substrates for methanogenesis, which creates CH_4-enriched biogas. With the AD process being sequential, the products of one stage are utilized as substrates in the subsequent stage. Thus in order to maintain a stable digester, the metabolic activity of the consortium of bacteria must be relatively balanced. Utilizing a readily degradable substrate can result in rapid hydrolysis and VFA production, which can lower the pH of the digester if the buffering capacity of the system is exceeded. Acidic conditions adversely affect the metabolic functions of methanogens, resulting in suppressed CH_4 production (Gerardi, 2003; Bouallagui et al., 2005; Ward et al., 2008).

A key factor to consider for operating a stable digester with optimized CH_4 production is determining the appropriate inoculum to substrate ratio (ISR) particularly during start-up and in batch-loaded systems (Motte et al., 2013; Zhou et al., 2011). Inoculum provides acclimated anaerobic microorganisms to accelerate biogas production as well as alkalinity for improved buffering capacity (Gu et al., 2014). However, inoculum has low biogas potential due to its recalcitrance and previous digestion. Therefore, reducing the inoculum level in the digester will allow for the addition of more substrates that have higher biogas potential, but ample inoculum volume is needed for stable CH_4 production.

To date, results cited in the literature on the influence of the ISR on CH_4 production are highly variable. Dechrugsa et al. (2013) observed that increasing the ISR (g VS basis) of rubber latex digester inoculum and para-grass from 1.0 to 4.0 increased CH_4 production by 26% (from 370 to 466 L CH_4/kg TS added). Similarly, Wei et al. (2014) showed that increasing the ISR from 0.5 to 2.0, significantly increased biogas production for mono-digestion of barley straw, pig manure, and cattle manure. In contrast, Eskicioglu and Ghorbani (2011) determined that increasing the ISR of sewage treatment plant sludge inoculum and whole stillage from 0.46 to 3.67 decreased CH_4 production by 12% (from 458 to 401 L CH_4/kg VS). Similarly, Slimane et al. (2014) observed that with WWTP sludge inoculum and slaughterhouse waste that increasing the ISR from 0.3 to 1.0 decreased biogas production by more than 40% (from 864 to 504 mL). Zhou et al. (2011) observed that optimal CH_4 production occurred within a narrow range of ISRs, with the highest specific CH_4 yield for digestion of municipal WWTP sludge inoculum and fresh okara occurring at an ISR (VS basis) of 1.67 (495 L CH_4/kg

VS), which was also found to be similar to the specific CH_4 yields observed at ISRs 1.11 – 1.43 (478 – 490 L CH_4/kg VS). However, increasing the ISR from 1.67 to 10, decreased CH_4 production to 183 L CH_4/kg VS, while decreasing the ISR from 1.11 to 0.33 significantly reduced the CH_4 yield to 8 L CH_4/kg VS (Zhou et al., 2011). The review of literature suggests that the effect of the ISR on CH_4 production varies depending on the substrate and inoculum source.

Dairy farmers are increasingly growing cover crops (Bryant et al., 2013), such as forage radish, to achieve multiple soil and environmental benefits (Chen and Weil, 2010; Chen and Weil, 2011; Lawley et al., 2012; Weil et al., 2009). Forage radish cover crops, which would otherwise winter-kill, have the potential to be utilized as an AD co-substrate to increase biogas production of a manure-based digester during the fall and winter months, when the demand for supplemental heating is the highest. The benefits of the cover crop on the field for increased topsoil fertility and soil compaction alleviation are retained, as only the above-ground radish biomass is harvested for digestion. Our previous research has shown that utilizing the above-ground radish biomass as a co-substrate significantly increased CH_4 production of a dairy manure-based digester (Belle et al., 2015). As a positive linear relationship was observed between radish content in the co-digestion mixture and CH_4 production when inoculum was not a limiting factor, the goal of this research was to investigate the influence of the ISR on CH_4 production as the inoculum level was reduced. To our knowledge, no literature is available regarding this effect when utilizing radish cover crops as a co-digestion substrate with dairy manure or when digested alone. The specific objectives of this research were to: (1) determine the effect of the ISR on CH_4 production when digesting only forage radish cover crops and when co-digesting radish with dairy manure, (2) determine the effect of substrate composition on CH_4 production, and (3) determine the effect of VFA production and pH on CH_4 production at varying ISRs.

3.3 Materials and Methods

3.3.1 Feedstocks

Forage radish cover crops (*Raphanus sativus* var. *longipinnatus*) and dairy manure (liquid fraction of solids-separated manure) were used as digestion substrates. The above-ground biomass of the forage radish cover crop was harvested from a USDA facility located in Beltsville, MD (39.03°, -76.89°). Planting of the radish cover crop occurred in August immediately after corn silage harvest. The radish was harvested by hand prior to winter-kill from randomized 1 m^2 quadrants. A stainless steel knife was used to harvest the above-ground biomass, which consisted of the leafy shoots plus a small portion of the fleshy root that extended above the soil surface. Each radish was cut approximately 3 to 5 cm from the soil surface and frozen in heavy-duty plastic bags until use. After thawing, a food processor was used to chop the radish into a semi-slurry material in order to simulate harvesting the radish in the field utilizing a rotary mower and forage chopper (Belle et al., 2015).

Dairy manure was obtained from the 120-cow USDA research dairy facility. The dairy manure was scraped and stored in a manure pit prior to solids separation with a FAN separator, which removes roughly 80% of the solids. The liquid fraction of the separated manure is treated in a mesophilic (25-35°C) complete-mix anaerobic digester

(540 m^3). Inoculum from this digester was obtained from a sampling port located inside the digester and was utilized in this batch study.

3.3.2 Experimental design

To determine the influence of the ISR on CH_4 production, a biochemical methane potential (BMP) experiment was conducted based on a modified method of Moody et al. (2011). Two substrates were utilized, radish cover crops only and a co-digestion mixture containing 40% radish and 60% dairy manure, by wet weight (ww). The BMP experiment was conducted using 36 glass serum bottles (300 mL), with three replicates of 12 treatment groups. All feedstocks were added on a ww basis. The amount of substrate added to each bottle was kept constant. The inoculum was added at varying ratios, resulting in ISRs containing inoculum additions of 65%, 50%, 35%, 20%, 10%, and 0% (ww basis) for each substrate (Table 3.1). The ISRs ranged from 0 – 0.48 on a gram VS basis, with treatments containing the 40% radish co-digestion mixture as substrate having higher ISR values due to the substrate having lower VS content. The results were compared to our previous radish co-digestion study using an ISR containing 91% inoculum (ww), which was determined to be a non-limiting quantity of inoculum (data unpublished).

To account for biogas production from residual biodegradable material in the digester inoculum, triplicate controls containing only inoculum were incubated and sampled simultaneously to allow subtraction of biogas production not attributed to the substrates. Nutrient media was not utilized as dairy manure has been shown to contain the necessary micronutrients for digestion (Al Seadi et al., 2008).

The bottles were purged with N_2:CO_2 gas (70:30 vv) to displace residual oxygen and capped with a rubber septum to create an anaerobic environment. The bottles were placed on a continuous orbital shaker (New Brunswick Scientific; Edison, NJ USA; model Innova 2300) at 117 RPM and incubated in a darkened environmental chamber at 35^{o}C. The BMP was conducted for 60-days, which equaled the time period in which biogas production had largely ceased, with daily biogas production in the final 7 days of incubation contributing < 1% of the cumulative biogas production.

3.3.3 Biogas analysis

Biogas production and CH_4 content of the produced biogas were measured approximately daily for the first week, every other day during the second week of the study, bi-weekly, and weekly, with the frequency of biogas measurement based on the quantity of biogas produced. The produced biogas was quantified by volumetric displacement using a wetted glass gas-tight graduated syringe (50 mL) (Popper & Sons, Inc.; New York USA) inserted through the rubber septum of the BMP bottle into the gas headspace with pressure displacing the syringe plunger until equilibrium. The biogas was analyzed for CH_4 content using a gas chromatograph (Agilent Technologies, Inc.; Shanghai China; model 7890 A) with a thermal conductivity detector (TCD) at 250°C, an HP-Plot Q capillary column (Agilent J&W; USA), and He as the carrier gas at 8.6 ml/min. The oven operated at 60°C for 2 min and subsequently ramped at 30°C/min to 240°C.

Table 3.1 Feedstock loading for the inoculum to substrate ratio (ISR) study.

	Inoculum *(g_{ww})*	**Radish** *(g_{ww})*	**Manure** *(g_{ww})*	**Inoculum** *(g VS)*	**Radish** *(g VS)*	**Manure** *(g VS)*	**Total** *(g VS)*
Substrate: 100% Radish							
ISR 65	37.14	20.00	0.00	0.52	1.66	0.00	2.18
ISR 50	20.00	20.00	0.00	0.28	1.66	0.00	1.94
ISR 35	10.77	20.00	0.00	0.15	1.66	0.00	1.81
ISR 20	5.00	20.00	0.00	0.07	1.66	0.00	1.73
ISR 10	2.22	20.00	0.00	0.03	1.66	0.00	1.69
ISR 0	0.00	20.00	0.00	0.00	1.66	0.00	1.66
Substrate: 40% Radish/60% Manure							
ISR 65	37.14	8.00	12.00	0.52	0.66	0.42	1.61
ISR 50	20.00	8.00	12.00	0.28	0.66	0.42	1.37
ISR 35	10.77	8.00	12.00	0.15	0.66	0.42	1.24
ISR 20	5.00	8.00	12.00	0.07	0.66	0.42	1.16
ISR 10	2.22	8.00	12.00	0.03	0.66	0.42	1.12
ISR 0	0.00	8.00	12.00	0.00	0.66	0.42	1.09

3.3.4 Feedstock characterization

The inoculum, dairy manure, and forage radish were characterized for pH, total solids (TS), and volatile solids (VS) (Table 3.2). The pH was determined with a glass electrode and an Accumet AB 15 pH meter. Standard Methods for the Examination of Water and Wastewater (APHA, 2005) were used to determine TS (Method 2540B) and VS (Method 2540 E). Each treatment was analyzed before and after digestion for pH and VFAs. For VFA determination, acidified (pH 2.0) samples were centrifuged for 20 min at 5,000 RPM and the supernatant filtered stepwise to 0.22 µm. The liquid filtrate was analyzed for VFA concentration using a gas chromatograph (Agilent Technologies, Inc.; Shanghai China; model 7890 A) with a flame ionization detector (FID) at 300°C, a DB-FFAP capillary column (Agilent J&W; USA), and He as the carrier gas at 1.80 ml/min. The injection temperature was held at 250°C and the oven operated at 100°C for 2 min and subsequently ramped at 10°C/min for a total run time of 10 min. The GC was equipped with an Agilent Technologies 7693 autosampler. Total volatile fatty acid (TVFA) concentrations are expressed as acetic acid.

Table 3.2 Characteristics of feedstocks.

	Inoculum	**Dairy Manure**	**Forage Radish**
pH	7.94 (0.01)	7.82 (0.01)	5.60 (0.01)
TS *(mg/g)*	22.5 (0.4)	46.6 (0.03)	94.4 (0.2)
VS *(mg/g)*	14.0 (0.1)	35.3 (0.03)	82.8 (0.1)

3.3.5 Statistical analysis

The experimental design was a completely randomized design. The 6x2 factorial treatment structure contained 2 factors, ISR and substrate composition, resulting in 12 replicated treatment combinations. A multi-way ANOVA was conducted using *SAS 9.3* (SAS, Cary, NC) to determine significant differences for average CH_4 yields. Due to a significant interaction between the two factors, contrast statements were conducted on simple effect means to make comparisons. The level of significance was held at 0.05 for all statistical analyses. Reported values are given as means with standard errors.

3.4 Results and Discussion

3.4.1 Methane production for the 40% radish co-digestion mixture

The highest specific CH_4 yield for a 40% radish co-digestion mixture was observed at an ISR containing 65% inoculum (284 ± 0.4 L CH_4/kg VS) followed by 50% inoculum (239 ± 4 L CH_4/kg VS) (Figure 3.1; Table D.1). Although a large volume of CH_4 was produced, reducing the inoculum from 65% to 50% (ww basis), significantly decreased CH_4 production (p-value < 0.001). These findings are comparable to those reported by Dechrugsa et al. (2013) where para-grass and pig manure co-digestion mixtures resulted in higher CH_4 production occurring at higher ISRs. An ISR containing 65% and 50% inoculum (ww basis) corresponded to an ISR of 0.48 and 0.26, respectively, on a VS basis (Table 3.3). Similar to results from Kawai et al. (2014), increasing the ISR (VS basis) from 0.25 to 0.50, increased CH_4 production (162 – 236 L CH_4/kg VS). However in their study utilizing food waste (low labile organic fraction) as substrate and sewage sludge inoculum produced 17% – 32% less CH_4 than observed with radish co-digestion. Overall, with a 40% radish co-digestion mixture, decreasing the ISR below 50% inoculum adversely effected CH_4 production, with zero to negligible CH_4 production observed at ISRs containing $\leq$ 35% inoculum. The ISRs containing $\leq$ 35% inoculum corresponded to an ISR of $\leq$ 0.14 on a VS basis and comparisons of CH_4 yields could not be made to other studies as ISRs (VS basis) were $\geq$ 0.25 in the literature.

With an ISR containing 65% inoculum, 91% of the cumulative CH_4 production occurred the first 28 days of the 60-day incubation period (Figure 3.1). Methane production occurred immediately on Day 1, reaching a maximum CH_4 production rate on Day 5, followed by a steady decrease in the production rate throughout the remaining incubation period. The CH_4 production rate was lower as the ISR was reduced to 50% inoculum. Similarly, Kawai et al. (2014) observed that the lag in CH_4 yield increased as the ISR decreased. For an ISR containing 50% inoculum, the maximum CH_4 production rate was not observed until Day 21 (Figure 3.1). After Day 21, the CH_4 production rate remained relatively stable until after Day 46, when production began to taper off. In addition, the ISR containing 50% inoculum had the most variability during the period of high CH_4 production, suggesting that within each triplicate bottle there may have been digestion conditions that affected CH_4 production at varying rates among the bottles. However cumulative CH_4 production values of the triplicate bottles were similar by Day 60.

The CH_4 concentration of the biogas (61 ± 0.5%) was stable after Day 11 digesting with an ISR containing 65% inoculum. However for 50% inoculum, the CH_4 concentration gradually increased over time, surpassing the CH_4 concentration of the

65% inoculum treatment by Day 39, averaging 65 ± 0.7% from Days 39 – 60 (Figure 3.1).

Table 3.3 Average cumulative CH_4 production at varying ISRs.

Substrate	**40% Radish/60% Manure**		**100% Radish**	
	ISR[b]	*L CH_4/kg VS*	*ISR*[b]	*L CH_4/kg VS*
ISR 65[a]	0.48	284 (0.4)	0.31	6.15 (0.91)
ISR 50	0.26	239 (4)	0.17	0.67 (0.05)
ISR 35	0.14	2.46 (0.03)	0.09	0.02 (0.02)
ISR 20	0.06	0.86 (0.08)	0.04	0 (0)
ISR 10	0.03	0.48 (0.03)	0.02	0 (0)
ISR 0	0	0.03 (0.01)	0	0 (0)

[a] Inoculum to substrate ratio (ISR) containing 0-65% inoculum (ww basis).
[b] VS basis
Values are means (± standard error) from triplicate samples.

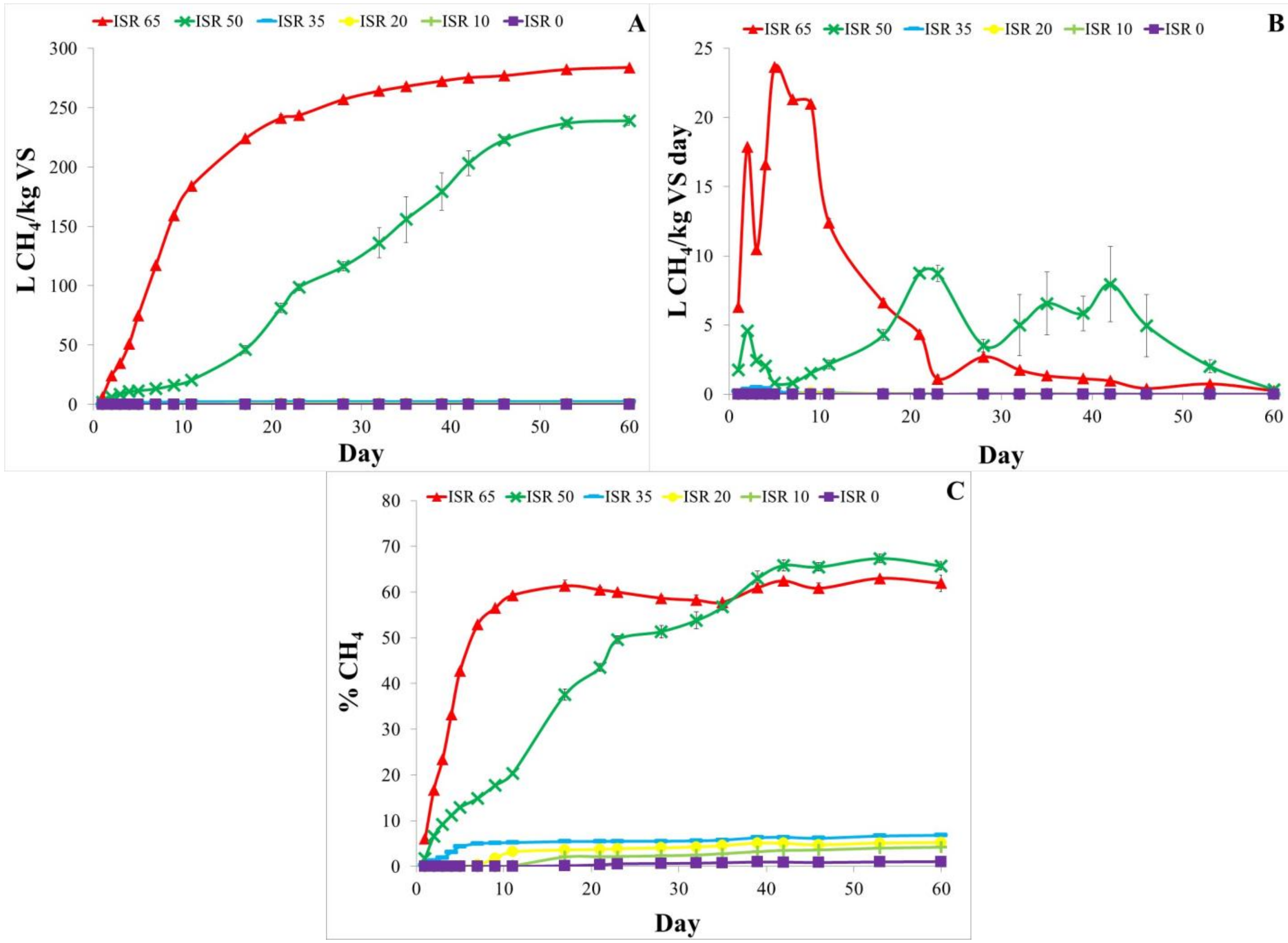

Figure 3.1 Average (± standard error) CH_4 production from 40% radish (ww) co-digested with dairy manure at different inoculum to substrate ratios (ISR, by ww). **(A)** L CH_4/kg VS of substrate added, **(B)** daily CH_4 production rate, and **(C)** CH_4 content in the biogas.

3.4.2 *Methane production for 100% radish*

Increasing the ISR also increased CH_4 production when utilizing 100% radish as substrate (Figure 3.2). However, the specific CH_4 yield for 100% radish at an ISR containing 65% inoculum (6.15 ± 0.91 L CH_4/kg VS) was 98% lower compared to 40% radish as substrate.

With 65% inoculum, CH_4 production started immediately on Day 1, with the maximum CH_4 production rate occurring on Day 2 followed by a rapid decline in CH_4 production. By Day 4, the daily rate of CH_4 production was < 1 L CH_4/kg VS.day. The CH_4 concentration of the biogas was also 46% lower than with co-digestion, averaging 15 ± 0.2% during incubation (Figure 3.2). Below an ISR containing 65% inoculum, the specific CH_4 yields were < 1 L CH_4/kg VS. Utilizing 65% inoculum and 100% radish as substrate, on a VS basis the ISR (0.31) was in the ISR range of 0.26 and 0.48 (ISRs containing 50 and 65% inoculum, respectively, with 40% radish as substrate). However CH_4 yields were drastically lower utilizing 100% radish as substrate, further highlighting that substrate composition as well as the quantity of inoculum affects CH_4 production (Table 3.3).

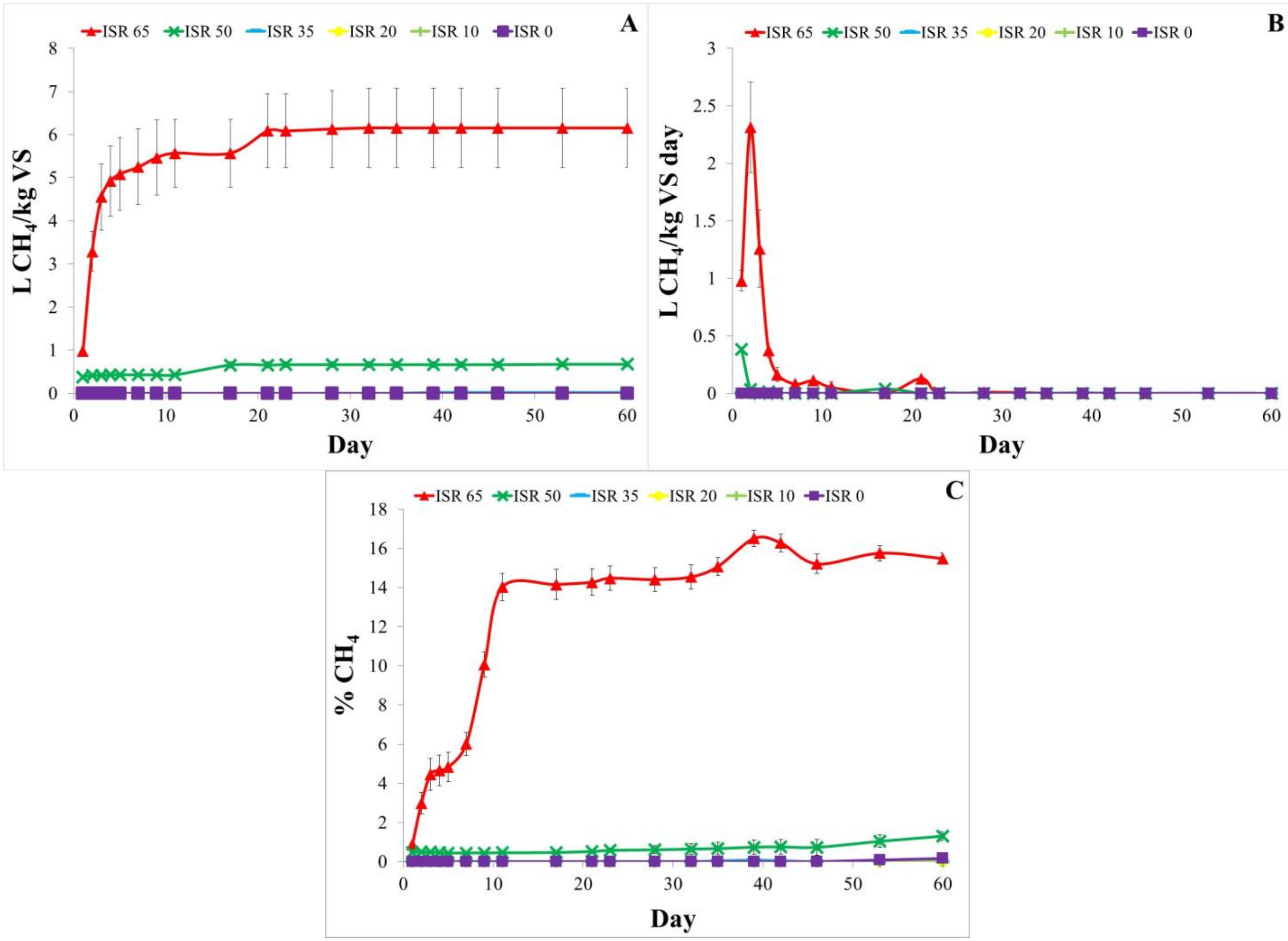

Figure 3.2 Average (± standard error) CH_4 production from 100% radish at different inoculum to substrate ratios (ISR, by ww). **(A)** L CH_4/kg VS of substrate added, **(B)** daily CH_4 production rate, and **(C)** CH_4 content in the biogas.

Overall, the statistical output reveals that there was a significant interaction between ISR and substrate composition (p-value < 0.001). There was a significant difference in CH_4 production, with higher inoculum additions producing more CH_4. However, the magnitude of increase in CH_4 production was dependent on substrate composition, with the 40% radish co-digestion mixture producing significantly more CH_4 than 100% radish at an ISR containing 65% inoculum (p-value < 0.001). Thus, in order to obtain enhanced CH_4 production, it is recommended to not reduce the ISR below 65% inoculum when digesting 40% radish in a dairy manure-based digester.

3.4.3 Co-digestion and 100% radish at an ISR with 91% inoculum

In a prior study, it was determined that increasing the radish content of a dairy manure co-digestion mixture increased CH_4 production when utilizing an ISR containing 91% inoculum. In that study, the highest specific CH_4 yield was observed with 100% radish (372 ± 12 L CH_4/kg VS), while the 40% radish co-digestion mixture produced 345 ± 2 L CH_4/kg VS (data unpublished). These CH_4 yields for 100% and 40% radish were greater than the CH_4 yields observed when digesting with an ISR containing 65% inoculum, the highest ISR tested in the current study. This finding suggests that increasing the ISR beyond 65% inoculum continues to increase CH_4 production (Figure 3.3). With 100% radish, increasing the ISR from 65% to 91% inoculum had a greater effect on CH_4 production (366 L CH_4/kg VS) than what was observed utilizing the 40% radish co-digestion mixture as substrate (61 L CH_4/kg VS).

For 40% radish, between the ISRs containing 50% and 91% inoculum, there appears to be a strong positive linear relationship (R^2= 0.996) between inoculum quantity and CH_4 production. A similar relationship could not be determined for 100% radish as substrate, but the data does suggest that a large amount of inoculum > 65% is required for enhanced CH_4 production, especially when operated in batch mode. Overall, although there was a significant difference in CH_4 yields for 40% radish and 100% radish when utilizing an ISR containing 65% inoculum, increasing the ISR to 91% inoculum, CH_4 production was found to be statistically similar. These results are similar to those reported by Dechrugsa et al. (2013), where combining higher ISRs with higher para-grass mix ratios yielded higher CH_4 production. Pig farm digestate inoculum and 100% para-grass had the highest CH_4 yield, with increasing the ISR from 1.0 to 4.0 increasing CH_4 yield from 332 to 522 L/kg TS, with the optimal ISR occurring between 3.0 and 4.0.

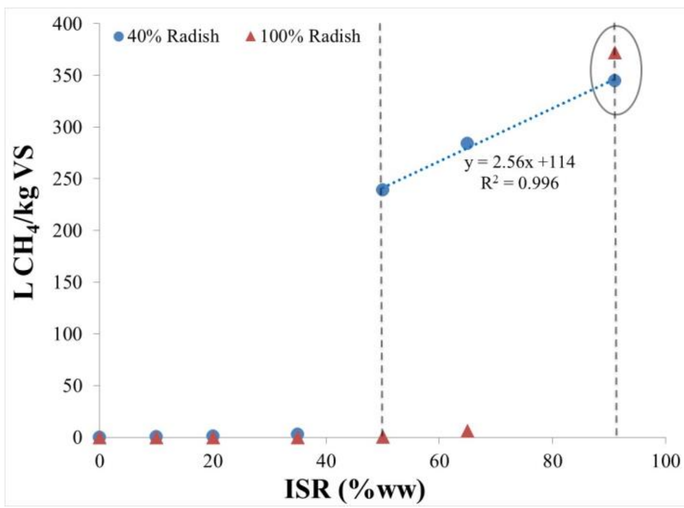

Figure 3.3 Relationship between inoculum to substrate ratio (ISR) and CH_4 production. Data points at an ISR containing 91% inoculum obtained from a prior study, with regression data from inside the dashed lines of 50 to 91% ISR.

3.4.4 Effect of volatile fatty acids and pH on methane production

The 40% radish co-digestion mixtures had an average influent TVFA concentration of 5.23 ± 0.30 g/L pre-digestion, while the 100% radish mixtures had a lower average TVFA concentration (0.55 ± 0.09 g/L) pre-digestion. The difference in

TVFA concentrations is due to the liquid manure contributing soluble VFAs while the solid radish substrate was not solubilized pre-digestion.

The pH values of the 40% radish co-digestion treatments pre-digestion ranged from 6.82 – 7.55, with treatments containing a larger quantity of inoculum having higher pH values (Figure 3.4). The 100% radish treatments had lower pH values, ranging from 5.60 – 7.15 (Figure 3.5), due to inclusion of more radish substrate, which has an average pH of 5.6. The additional buffering capacity of the manure also likely contributed to the higher pH values of the 40% radish co-digestion treatments. No pH or alkalinity adjustments were made prior to digestion. The optimal pH range for mesophilic digestion is 6.5 – 8.0. All treatments were within the optimal pH range pre-digestion, except the 100% radish treatments at an ISR containing less than 20% inoculum, where pH values were below the desired pH range (pH $\leq$ 6.25).

Post-digestion, VFAs were not detected in the 40% radish treatments when the ISR contained $\geq$ 50% inoculum (Figure 3.4), indicating that the VFAs were consumed by the methanogens, as high volumes of CH_4 were produced (239 – 284 L CH_4/kg VS) and pH values remained within the optimal range. However, the 40% radish treatments post-digestion with an ISR containing $\leq$ 35% inoculum had an accumulation of VFA production (13.0 – 15.6 g TVFA/L), with pH values dropping below 6.0 and negligible CH_4 production (< 3 L CH_4/kg VS). The ISRs containing $\leq$ 35% inoculum had on average an acetic to propionic acid ratio of 1.5, with a decrease in the inoculum proportion leading to an increase in butyric (1.77 – 3.27 g/L) and valeric acid (1.17 – 2.24 g/L) production.

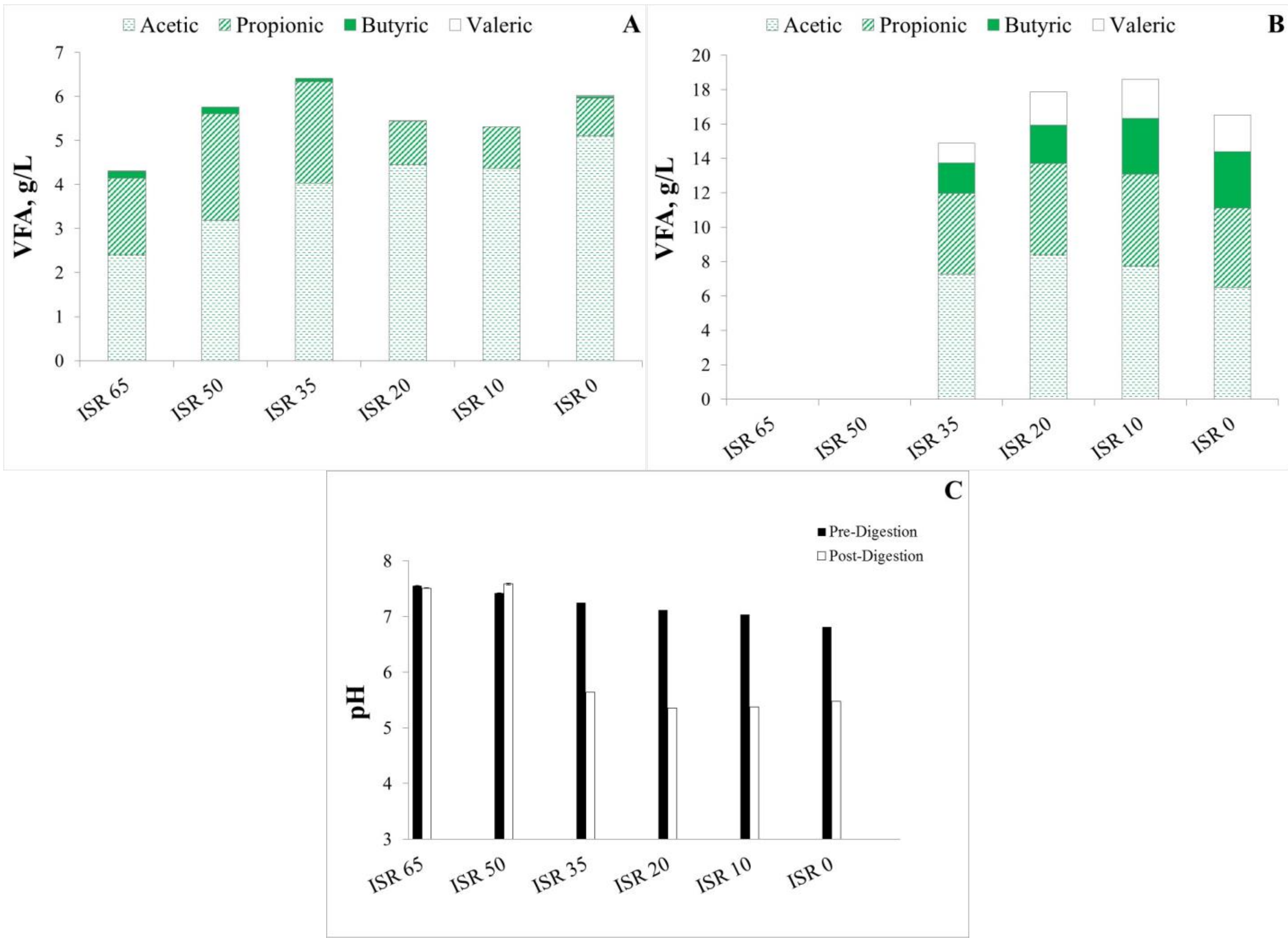

Figure 3.4 Characteristics of 40% radish (ww) co-digested with dairy manure at different inoculum to substrate ratios (ISR). Panels represent: **(A)** pre-digestion VFA concentrations, **(B)** post-digestion VFA concentrations, and **(C)** pH values.

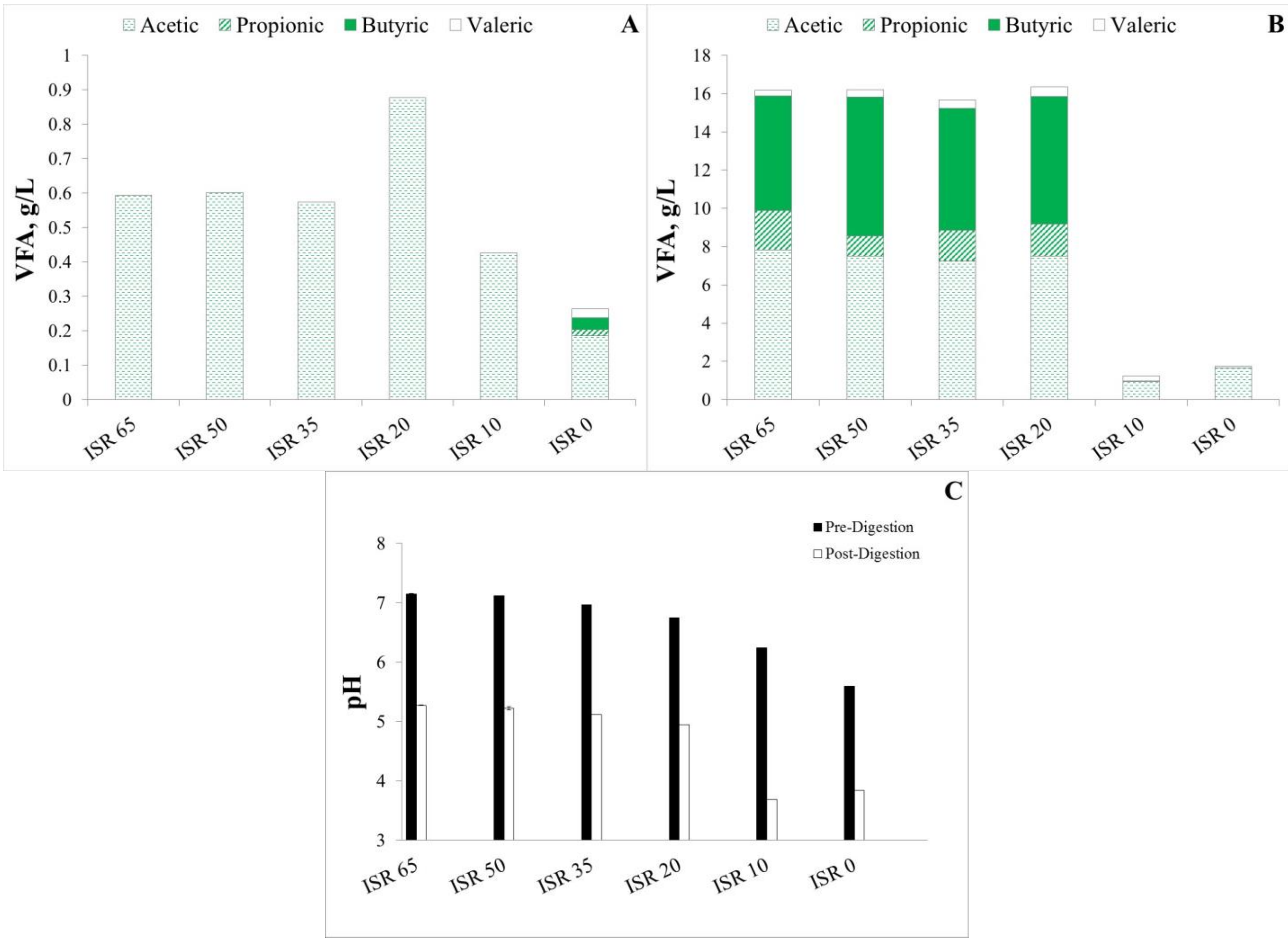

Figure 3.5 Characteristics of 100% radish at different inoculum to substrate ratios (ISR). Panels represent: **(A)** pre-digestion VFA concentrations, **(B)** post-digestion VFA concentrations, and **(C)** pH values.

For the 100% radish treatments post-digestion, all ISRs had an accumulation of VFA production, averaging 13.5 ± 0.1 g TVFA/L (Figure 3.5). The ISRs containing ≤ 10% inoculum were not included in this assessment due to the small volume of liquid sample obtained for VFA determination, which may not have been representative of the treatment. The pH values of the 100% radish treatments post-digestion ranged from 3.69 – 5.27, with CH_4 production being adversely effected (< 7 L CH_4/kg VS) at each ISR. There was also an accumulation of butyric acid (5.99 – 7.26 g/L), with a decrease in the proportion of inoculum increasing valeric acid production post-digestion.

Comparing post-digestion VFA production utilizing a 40% radish co-digestion mixture and 100% radish as substrates at ISRs containing 35% and 20% inoculum, the acetic acid concentrations were similar (7.25 – 8.4 g/L). However, the 100% radish treatments had a larger increase in acetic acid production during digestion, likely due to the rapid hydrolysis of the radish substrate. Post-digestion, the acetic to propionic acid ratio was higher (4.47) in the 100% radish treatments, with three times more butyric acid production, but less valeric acid production than the 40% radish co-digestion treatments.

Overall, the data suggests that increased ISRs and utilizing manure as a co-substrate for radish digestion aids in buffering the digestion environment from changes in pH and circumvents souring of the digester as evidenced by the accumulation of VFAs. Butyric and valeric acid accumulation strongly corresponded with digester failure, with a butyric to valeric acid ratio > 1 (g/L basis) resulting in negligible CH_4 production (< 7 L CH_4/kg VS). Similarly, Zhang et al. (2013) observed that CH_4 production was more favorable when acidic substrates were co-digested with cattle manure and suggested that the alkalinity of cattle manure may help to buffer the system when pH adjustments are not utilized in the batch system. Furthermore, Jiang et al. (2012) demonstrated that the addition of cattle slurry to vegetable waste (pH 5.77) improved digester performance, with accumulated VFAs consumed over time.

There are several possible reasons for CH_4 suppression as the quantity of inoculum was reduced in the batch system. By reducing the inoculum quantity, the quantity of methanogens present may not have been sufficient. Methanogens have a slow generation time (Wellinger et al., 2013). As the population increases over time, the rate of CH_4 production increases. When the ISR was reduced from 65% to 50% inoculum in the radish co-digestion mixture, there appeared to be adequate consumption of VFAs and H_2 initially, although at a slower rate, which prevented the accumulation of acids and low pH. However, when the ISR was reduced below 50% inoculum, even with co-digestion, VFAs accumulated in excess of the buffering capacity of the system, as pH values declined with suppressed methanogen metabolic activity observed.

While the methanogen population could have been abundant initially in the 100% radish treatment at an ISR containing 65% inoculum, rapid hydrolysis of the readily degradable radish substrate and subsequent VFA production could have occurred at a faster rate than the methanogens were able to utilize the substrates. If acetate and H_2 accumulated in the digester, the rate of acetogenesis would likely decrease due to acetogenic bacteria favoring a low hydrogen partial pressure (Wellinger et al., 2013). With suppressed acetogenesis, an accumulation of longer chain fatty acids, such as butyric and valeric acids, could occur as VFA conversion into acetate diminishes. As methanogens were unable to directly utilize these longer chain fatty acids prior to conversion into acetate, the buffering capacity of the system was likely exceeded (Gerardi, 2003).

3.5 Conclusions

Anaerobic digestion of two substrates, 100% forage radish and 40% radish co-digested with dairy manure, was investigated at varying ISRs. A key finding from this research was that as the quantity of radish was increased in the digester, a larger quantity of inoculum was required during batch digestion to achieve enhanced CH_4 production. This trend was verified using VFA analyses. However, by co-digesting the forage radish with a substrate such as dairy manure, which also has methanogens and alkalinity, the quantity of inoculum added to the digestion system could be greatly reduced. Digestion instability was observed for each substrate at ISRs containing ≤ 35% inoculum, resulting in an accumulation of VFAs, low pH values (≤ 5.64), and negligible CH_4 production.

3.6 Acknowledgements

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Chapter 4: Anaerobic Co-digestion of Forage Radish and Dairy Manure in Complete Mix Digesters

Ashley J. Belle, Stephanie Lansing, Walter Mulbry, and Ray R. Weil

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4.1 Abstract

Pilot-scale digesters (850 L) were used to quantify CH_4 and H_2S production when using forage radish cover crops as a co-digestion feedstock in dairy manure-based digesters. During two trials, triplicate mixed digesters were operated in batch mode with manure-only or radish + manure (27% and 13% radish by wet weight in Trial 1 and 2, respectively). Co-digestion increased CH_4 production by 11% and 39% in Trial 1 and 2, respectively. As H_2S production rapidly declined in the radish + manure digesters, CH_4 production increased reaching high levels of CH_4 ($\geq$ 67%) in the biogas. Over time, radish co-digestion lowered the H_2S concentration in the biogas (0.20%) beyond that of manure-only digestion (0.34 – 0.40%), although cumulative H_2S production in the radish + manure digesters was higher than manure-only. Extrapolated to a farm-scale (200 cows) continuous mixed digester, co-digesting with radish could generate 3150 m^3 CH_4/month, providing a farmer additional revenue up to $3125/month in electricity sales.

4.2 Introduction

Dairy manure has a low energy density in comparison to other anaerobic digestion (AD) feedstocks and economic returns from manure-only digestion are often low to negative (Klavon et al., 2013; Wang et al., 2011). However, biogas production from dairy digesters can become a more economically viable practice by using additional biodegradable feedstocks located in close proximity to the dairy facility (El-Mashad and Zhang, 2010). Co-digesting dairy manure with other substrates, such as fats, oils, and grease (Lansing et al., 2010), slaughterhouse waste (Alvarez and Linden, 2008), or energy crops (Amon et al., 2007) with higher biogas potential have been shown to increase biogas production by 100 – 500% (El-Mashad and Zhang, 2007; Lansing et al., 2010), thus increasing the feasibility of AD technology, especially for small to mid-sized dairy farmers (Klavon et al., 2013). Energy crop digestion is increasingly utilized due to the higher methane (CH_4) yield relative to animal manure (120 – 300 L CH_4/kg VS) (Al Seadi et al., 2008; Lansing et al., 2010). Some of the most widely used energy crops are maize (205 – 450 L CH_4/kg VS) (Bruni et al., 2010; Braun et al., 2009), switchgrass (298 – 467 L CH_4/kg VS) (Masse et al., 2010; Braun et al., 2009), sugar beets (236 – 381 L CH_4/kg VS) (Umetsu et al., 2006; Braun et al., 2009), sunflower grass (154 – 400 L CH_4/kg VS), and Sudan grass (213 – 303 L CH_4/kg VS) (Amon et al., 2007; Braun et al., 2009).

Forage radish is listed as a top cover crop species for the Northeast and Mid-Atlantic regions of the United States (SARE, 2007), with over 15,000 acres planted in Winter 2012 in the state of Maryland alone. Traditionally, corn-silage based dairy farmers leave the land fallow after harvesting corn silage in August. Planting forage radish as a winter cover crop immediately after corn harvest will not interfere with food supply. After several consecutive nights with temperatures below -6°C, the radish cover crop winter-kills and rapidly decays, leaving behind a clean and enhanced seedbed. Utilizing radish as a winter cover crop results in multiple soil and environmental benefits, such as erosion control, improved soil fertility, and the alleviation of soil compaction (Gruver et al., 2014).

Forage radish is a Brassica crop and has a relatively high sulfur content (7.8 – 8.2 g/kg dry matter in the shoots) (Lounsbury, 2013). During AD, organic sulfur decomposition leads to hydrogen sulfide (H_2S) production, as sulfate reducing bacteria

(SRB) utilize acetate to reduce SO_4^{2-} to H_2S. The most common pathway for CH_4 production is the acetoclastic pathway, where methanogens split acetate to form CH_4 and CO_2. Since SRB and methanogens both utilize acetate and H_2 as primary substrates, competition for available resources can occur during AD (Liu and Whitman, 2008). As sulfate reduction is more energetically favorable (-152 kJ/mol) than methanogenesis (CO_2 reduction: -131 kJ/mol; acetoclastic pathway: -37 kJ/mol) (Madigan et al., 2012), CH_4 production can be suppressed when H_2S production is high due to substrate limitation (Liu and Whitman, 2008). For example, Khanal and Huang (2003) found that during anaerobic treatment of high-sulfate wastewater, increasing sulfate concentration from 1,000 to 5,000 mg/L significantly increased H_2S production and decreased CH_4 production by 50%.

Hydrogen sulfide reacts with water vapor present in the biogas producing sulfuric acid, which can corrode piping and engine units. The H_2S content of biogas generated from animal manure ranges from 1000 to 3000 ppm (0.10 – 0.30%) (Al Seadi et al., 2008). The end use of the biogas dictates the extent to which the H_2S must be removed prior to usage. In combined heat and power (CHP) engines, the H_2S concentration should not exceed 100-500 ppm (0.01 – 0.05%) to prevent corrosion (Deublein and Steinhauser, 2011). Considering that biogas engines are often the largest cost associated with AD systems (Klavon et al., 2013) and that the majority of AD systems in the U.S. use the produced biogas for CHP or sole electricity production (AgSTAR, 2013), it is imperative that the H_2S concentration is not drastically increased when digesting sulfur-rich feedstocks, such as forage radish.

A major barrier to AD installation in the U.S. has been the lack of data on biogas potential in the literature, especially at the field-scale level. The data that is available in the literature focuses primarily on CH_4 production, with less attention to the effect of feedstock selection on H_2S production. This research investigates forage radish cover crops as a renewable source of energy in terms of CH_4 production, the effect of radish co-digestion on H_2S production, and the relationship between H_2S production and methanogenesis limitations. Specifically, this research seeks to determine if additional benefits can be obtained from the forage radish by harvesting the above-ground material prior to winter kill and utilizing it as a co-substrate in dairy digesters to increase CH_4 production. The overall objectives of this research were: (1) to determine potential CH_4 and H_2S production when co-digesting forage radish cover crops with dairy manure in batch pilot-scale complete mix digesters, (2) to determine how the percentage of forage radish in the co-digestion mixture affects CH_4 production, and (3) to quantify the radish crop acreage required for co-digestion at the farm-scale level and how inclusion of radish cover crops affects on-farm energy production potential. The results can be used by dairy farmers to maximize CH_4 production in digesters during the winter when the demand for supplemental heating is the greatest.

4.3 Materials and Methods

Pilot-scale complete mix anaerobic digesters were designed and constructed to evaluate the anaerobic co-digestion of forage radish (*Raphanus sativus* var. *longipinnatus*) and the liquid fraction of solids-separated dairy manure under field conditions. The research was conducted at the USDA Beltsville Agricultural Research Center (BARC) dairy farm (39.03°, -76.89°) located in Beltsville, Maryland.

4.3.1 Feedstocks

Forage radish was grown as a cover crop immediately after corn silage harvest, sown in August and harvested in early December prior to a predicted hard freeze. To harvest, the cover crop was first mowed with a rotary mower as close to ground level as practical (3 – 5 cm). This mow cut the leafy shoots plus a portion of the fleshy root that extended above ground into a windrow. A forage chopper then passed over the windrow to harvest the above-ground biomass and the chopped material was blown into an adjacent wagon for collection. During this process, the radish biomass was transformed into a semi-slurry state. The harvested forage radish biomass was stored in sealed plastic buckets and frozen until use. Due to the heterogeneity of the harvested radish particle size (up to 10 cm), an industrial vertical cutter (Hobart Corporation; Troy, Ohio USA; model VCM-40) was used to further reduce the radish particle size to less than 3 cm to prevent damaging the pumping system and clogging the digester piping during digester loading.

Solids-separated dairy manure was obtained from BARC's dairy research unit. This 120-cow dairy uses a scrape system to collect raw manure and a FAN separator (0.64 cm mesh screen) to remove roughly 80% of the solids prior to treatment in a mesophillic complete mix digester (540 m^3). The solids-separated dairy manure was collected from the holding pit on three different dates for the experiments. Total solids content of three sampling events varied 2 – 4%, primarily due to differences in water usage in the barn. Inoculum used in the batch digesters to accelerate biogas production was obtained from a sampling port located inside the BARC complete mix digester. The BARC digester is fed daily with the liquid fraction of solids-separated dairy manure and operates at 25 – 35°C. The inoculum substrate from inside the BARC digester had an average pH of 7.5, and total solids (TS) and volatile solids (VS) concentration of 24 and 15 mg/g, respectively (Table 4.1).

4.3.2 Experimental design

Six pilot-scale complete mix anaerobic digesters were fabricated from 850 L (working volume) high-density polyethylene conical tanks (Ace Roto-Mold; Hospers, Iowa USA) equipped with silicone adhesive rubber heating blankets (BriskHeat; Columbus, Ohio USA; model SRP series) and radiant foil shells to maintain a 35°C digestion temperature. Custom made top-mounted stirrers (using 1/15 hp Dayton right angle gear motors driving 25 cm diameter beveled mixing blades at 22 rpm) were used to agitate the contents twice daily for 15-minute periods. Two field trials were conducted using six digesters operating in batch mode for 33 days, which corresponded to the time period in which large decreases in daily biogas production were observed, with < 1% of the cumulative biogas production being produced daily. After the first 33-day trial, the six digesters were emptied and cleaned before being refilled for the second trial. For each trial, all digesters were loaded on the same day with the exception of the manure-only controls in Trial 1, which were loaded the following day. The digesters were operated in batch mode. All digesters contained 776 kg of total feedstock (Table 4.2). The dairy's truck scale (± 20 lbs) was used to weigh all feedstocks into a secondary container. Previous experiments demonstrated that the radish had a tendency to settle out of the manure. Therefore, the feedstocks were manually stirred in the secondary container while being transferred with a 1.5 hp centrifugal pump (Dayton; China) into each digester.

Table 4.1 Feedstock characterization represented by average (± standard error).

	M1		RM1 (27% radish:73% manure)			M2 and RM2 (13% radish:87% manure)		
	Inoculum	*Manure*	*Inoculum*	*Manure*	*Radish*	*Inoculum*	*Manure*	*Radish*
pH	7.5 (0.03)	6.9 (0.02)	7.5 (0.02)	6.9 (0)	4.6 (0.1)	7.5 (0.01)	7.2 (0.01)	4.3 (0.01)
sCOD *(g/L)*	3.95 (0.01)	11.9 (0.03)	4.08 (0.07)	12.8 (0.03)	51.9 (0.3)	3.15 (0.05)	8.03 (0.14)	51.2 (0.1)
TS *(mg/g)*	25.7 (0.1)	30.8 (0.4)	25.6 (0.02)	35.2 (0.02)	112 (4)	20.6 (0.1)	23.6 (0.1)	106 (0.4)
VS *(mg/g)*	16.0 (0.1)	22.3 (0.3)	16.0 (0.01)	26.1 (0.1)	89.6 (3.2)	12.7 (0.1)	17.2 (0.1)	81.4 (0.3)

Table 4.2 Feedstock loading.

	M1	RM1 (27% radish:73% manure)	M2	RM2 (13% radish:87% manure)
Inoculum, kg_{ww}	411	411	411	411
Manure, kg_{ww}	365	268	365	317
Radish, kg_{ww}	0	97	0	49
Total, kg_{ww}	776	776	776	776
Inoculum, kgVS	6.58	6.58	5.34	5.34
Manure, kgVS	8.04	6.98	6.21	5.39
Radish, kgVS	0	8.74	0	3.98
Total, kgVS	14.6	22.3	11.6	14.7
ISR[a]	1:1.2	1:2.4	1:1.2	1:1.8

[a] Inoculum to substrate ratio (ISR) calculated on a VS basis.

Results from previous laboratory-scale co-digestion experiments using dairy manure and forage radish mixtures showed that the optimal inoculum to substrate ratio (ISR) for radish and manure digestion was 65:35 on a wet weight (ww) basis (data unpublished). Due to differences in the VS content of the feedstocks during the laboratory and field trials, the ISR was adjusted for the pilot-scale experiment. All complete mix digesters were loaded in each field trial with 53% inoculum and 47% substrate (ww basis), with the substrate consisting of manure-only (control) or radish + manure (Table 4.2). Since the VS content of forage radish is over three times greater than dairy manure, the total amount of substrate (ww basis) added to each digester was kept constant to avoid substrate quantity being a confounding variable in the comparative study. As a result, the digesters had different VS contents. To make comparisons between treatments, the biogas data was normalized by the amount of VS added.

For Trials 1 and 2, three digesters contained manure-only (M1 and M2, respectively) and had an ISR of 1:1.2 (VS basis). For Trial 1, three radish + manure digesters contained 27% radish and 73% dairy manure (ww) (RM1), which corresponded to an ISR of 1:2.4. For Trial 2, the three radish + manure digesters (RM2) contained 13% radish and 87% dairy manure (ww), which corresponded to an ISR of 1:1.8 (Table 4.2).

4.3.3 Biogas analysis

The biogas generated was quantified with gas flow meters (EKM Metering Inc.; Santa Cruz, California USA; model EKM-PGM.75). Biogas samples were taken at least weekly from each digester with a syringe, placed into pre-evacuated foil gas bags, and analyzed for CH_4 and H_2S content using a gas chromatograph (Agilent Technologies, Inc.; Shanghai China; model 7890 A) with a thermal conductivity detector at 250°C with an HP-Plot Q capillary column (Agilent J&W; USA) and He as the carrier gas at 8.6 ml/min. The oven operated at 60°C for 2 minutes and subsequently ramped at 30°C/min to 240°C.

4.3.4 Feedstock characterization

The digester feedstocks were characterized prior to digestion (Table 4.1). Liquid digester samples were collected weekly and before and after each trial. Samples were analyzed for pH, soluble chemical oxygen demand (sCOD), total and volatile solids (TS, VS), total Kjeldahl nitrogen (TKN), total Kjeldahl phosphorus (TKP), and total sulfur. The pH was determined with an Accumet AB 15 pH meter. Standard Methods for the Examination of Water and Wastewater (APHA, 2005) were used to determine TS (Method 2540B) and VS (Method 2540E). The reactor COD digestion method was adapted by HACH Method 8000 for sCOD with 1.5 µm filtrate used for the analysis. A Lachat (QuickChem 8500 Series 2 FIA Automated Ion Analyzer) was used to determine TKN and TKP after Kjeldhal digestion with concentrated H_2SO_4 and $CuSO_4$*$5H_20$ and filtration through 0.45 µm membranes (QuikChem Method 13-107-06-2-D for TKN and 13-115-01-1-B for TKP). Composite samples from each digester type (manure-only or radish + manure) were submitted to Cumberland Valley Analytical Services (Hagerstown, MD) for total sulfur analysis and analyzed according to Standard Methods.

4.3.5 Statistical analysis

The experimental design for the two independent trials was a complete randomized design with six experimental units (digesters) and two treatment levels (presence or absence of radish) with three replicates each. A single factor ANOVA followed by Tukey-Kramer's post hoc test showed a significant difference between the CH_4 yields in the manure-only digesters (controls) in Trials 1 and 2. Therefore, statistical comparisons were only made within each trial. Significant differences within each trial were determined with t-tests for average CH_4 yields, H_2S yields, sCOD, TKN, and TKP using *SAS 9.3* (SAS, Cary, NC) with an alpha of 0.05. Reported values are given as means with standard errors.

4.4 Results and Discussion

4.4.1 CH_4 production

Co-digestion of forage radish cover crops in dairy manure complete mix batch digesters increased CH_4 production relative to that from digesters containing only dairy manure. The average CH_4 production value for RM1 (12.81 L CH_4/kg substrate) was 68% greater than M1 (7.61 L CH_4/kg substrate) (p-value = 0.003) and RM2 (8.38 L CH_4/kg substrate) was 77% greater than M2 (4.74 L CH_4/kg substrate) (p-value = 0.001) when normalized by kilograms of substrate added (Figure 4.1; Table 4.3). RM2 (13% radish) contained half the radish content of RM1 (27% radish), thus having a reduced VS load in comparison to RM1.

Table 4.3 Cumulative CH_4 and H_2S production represented by average (± standard error).

	M1	**RM1**	**M2**	**RM2**
L CH_4/kg VS	190 (9)	210 (7)	150 (10)	208 (7)
L H_2S/kg VS	1.71 (0.06)	2.21 (0.05)	0.91 (0.04)	1.68 (0.02)
L CH_4/kg $substrate_{ww}$	7.61 (0.35)	12.8 (0.4)	4.74 (0.32)	8.38 (0.28)
L H_2S/kg $substrate_{ww}$	0.07 (0.002)	0.14 (0.003)	0.03 (0.001)	0.07 (0.0008)

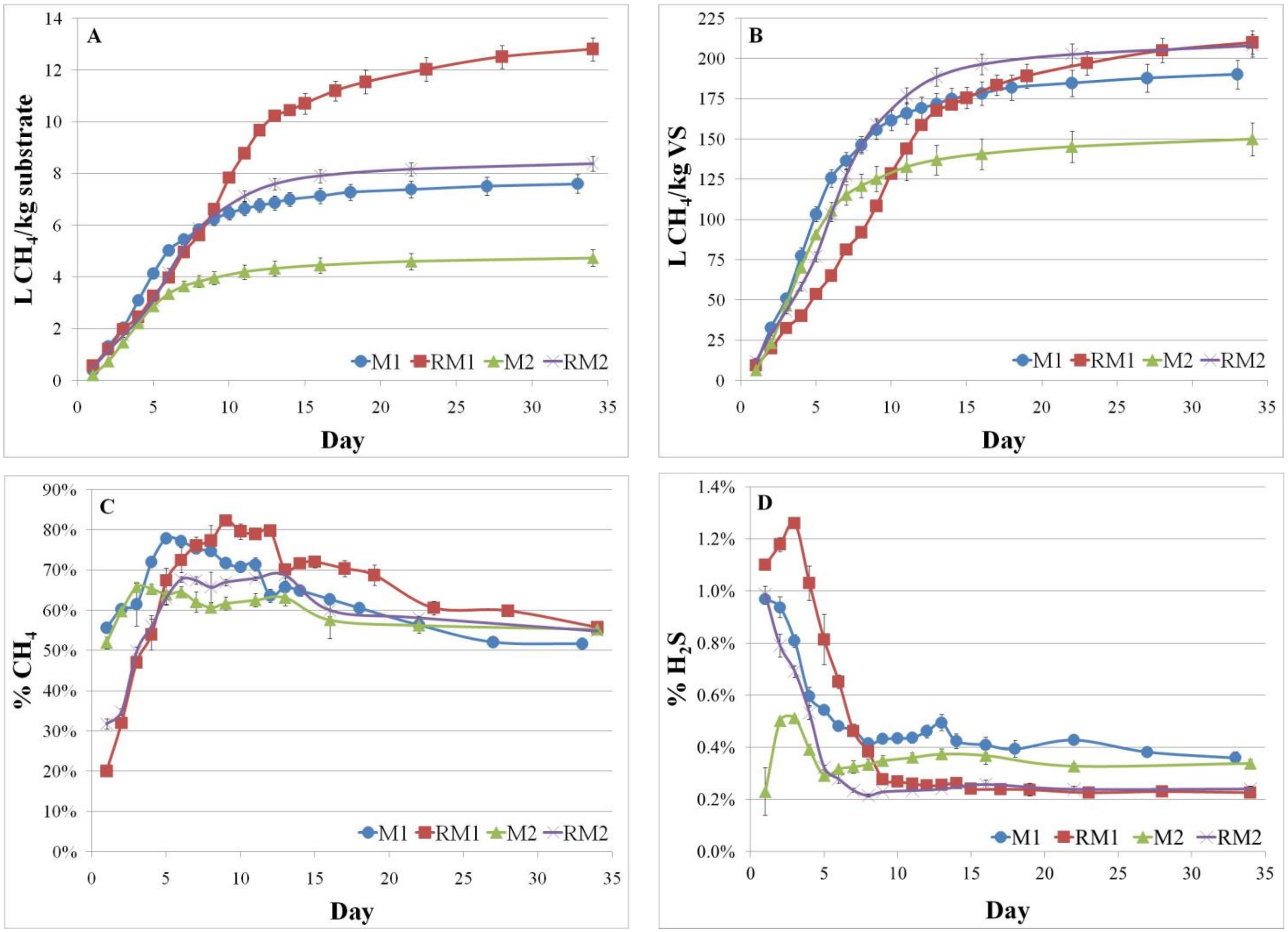

Figure 4.1 Average (± standard error) CH_4 and H_2S production for the manure-only (M1; M2) and radish + manure digesters (RM1: 27%; RM2: 13% radish, by ww). **(A)** L CH_4/kg substrate added, **(B)** L CH_4/kg VS added, **(C)** percent CH_4, and **(D)** percent H_2S.

The radish + manure digesters initially experienced a lag in CH_4 production compared to the manure-only digesters. Normalized by VS addition, RM1 and RM2 required approximately 15 and 7 days, respectively, to equal the CH_4 production of M1 and M2, respectively (Figure 4.1). Additionally, in Days 1 – 3, RM1 and RM2 had < 50% CH_4 in the biogas. However, by the time the digesters reached peak CH_4 production, the CH_4 concentration of RM1 and RM2 had increased to 76% and 67%, respectively. Overall, the CH_4 concentration of the biogas for RM1 and RM2 was significantly higher than the respective manure-only digesters: M1 and M2 (p-value = 0.012 and 0.045, respectively).

Although the dairy manure obtained for Trial 2 was more dilute due to increased use of misting units in the dairy barn, we expected that the CH_4 production values for M1 and M2 would be similar when normalized by VS added considering that each contained the same quantity of inoculum and manure (Figure 4.1; Table 4.2). In contrast to our expectations, average CH_4 production values from M1 (190 L CH_4/kg VS) and M2 (150 L CH_4/kg VS) were significantly different (p-value = 0.002). For this reason, differences in CH_4 production between the two trials could not be attributed only to changes in radish content and statistical comparisons could only be conducted within each trial. Although we cannot explain the basis for the differences in CH_4 production between M1 and M2, the more dilute manure in Trial 2 could have resulted in substrate limitations, or other factors such as nutrient availability or toxicity could have affected the digestion process.

When normalizing CH_4 production by VS, the CH_4 production values for M1 and RM1 differed by only 11% and were not statistically different (190 and 210 L CH_4/kg VS, respectively; p-value = 0.21). In Trial 2, there was a statistically significant difference between M2 and RM2, with a 39% increase in CH_4 production with radish (150 and 208 L CH_4/kg VS, respectively; p-value = 0.009) (Figure 4.1; Table 4.3). Although the effect of forage radish content (13% vs 27% radish) on CH_4 production was not statistically compared due to differences in manure between trials, the results show that even a small quantity of radish (13% radish) added to a dairy digester results in enhanced CH_4 production.

While co-digesting forage radish cover crops in dairy digesters increased CH_4 production, the CH_4 potential was lower than values from other energy crop digestion studies (i.e maize) but was similar to values from other sulfur-rich feedstocks. Amon et al. (2007) observed CH_4 production levels of 398 L CH_4/kg VS with maize, while Masse et al. (2010) observed 309 L CH_4/kg VS with switchgrass. Lower CH_4 production in our study (210 and 208 L CH_4/kg VS for RM1, RM2) is likely mostly due to co-digestion with manure, but could also be related to the higher sulfur content of the radish (0.6% DM) compared to the other energy crops utilized in these studies. The CH_4 potential of forage radish co-digestion was similar to other sulfur-rich biomass: seaweed (*Ulva sp.*) (148 L CH_4/kg VS), camelina (234 L CH_4/kg VS), and white mustard (223 L CH_4/kg VS) (Peu et al., 2011, 2012). Extrapolating from the pilot-scale studies, assuming no synergistic effects from co-digesting forage radish with dairy manure, digestion of 100% radish substrate would produce 515 L CH_4/kg VS. However, it is very likely that this value is overestimated, as our previous laboratory studies have shown that inoculum level (alkalinity) plays a major role in the digestion process as radish content increases (data unpublished).

4.4.2 Total sulfur content and H_2S production

During the first three days of incubation, there were elevated H_2S concentrations in the biogas produced in RM1 (1.2%), RM2 (0.8%), and M1 (0.9%), and a lower initial H_2S concentration in M2 (0.4%) (Figure 4.1). H_2S production rapidly declined during incubation as CH_4 production increased, likely due to the rapid utilization of the sulfur substrate over the first 14 days. By Days 9 and 7 respectively, RM1 and RM2 had lower H_2S concentrations (0.28% and 0.23%) compared to M1 and M2 (0.43% and 0.32%), and remained below the manure-only digesters throughout the rest of the 33-day digestion period.

The sulfur content of the radish used in this study was 0.65 mg S/g radish (ww basis). Our previous laboratory experiments showed the forage radish had twice the total sulfur content of dairy manure. The range for liquid dairy manure has been cited as 0.10 – 0.48 mg S/g manure (ww basis) (Page et al., 2014; Bao et al., 2010). The results show that there was no difference initially in total sulfur concentration between the manure-only and radish + manure digesters (Figure 4.2). It is likely that the sulfur concentrations were similar due to the relatively small proportion of radish (13% and 6% of total volume for RM1 and RM2, respectively) added to the radish + manure digesters in comparison to the proportion of inoculum and manure.

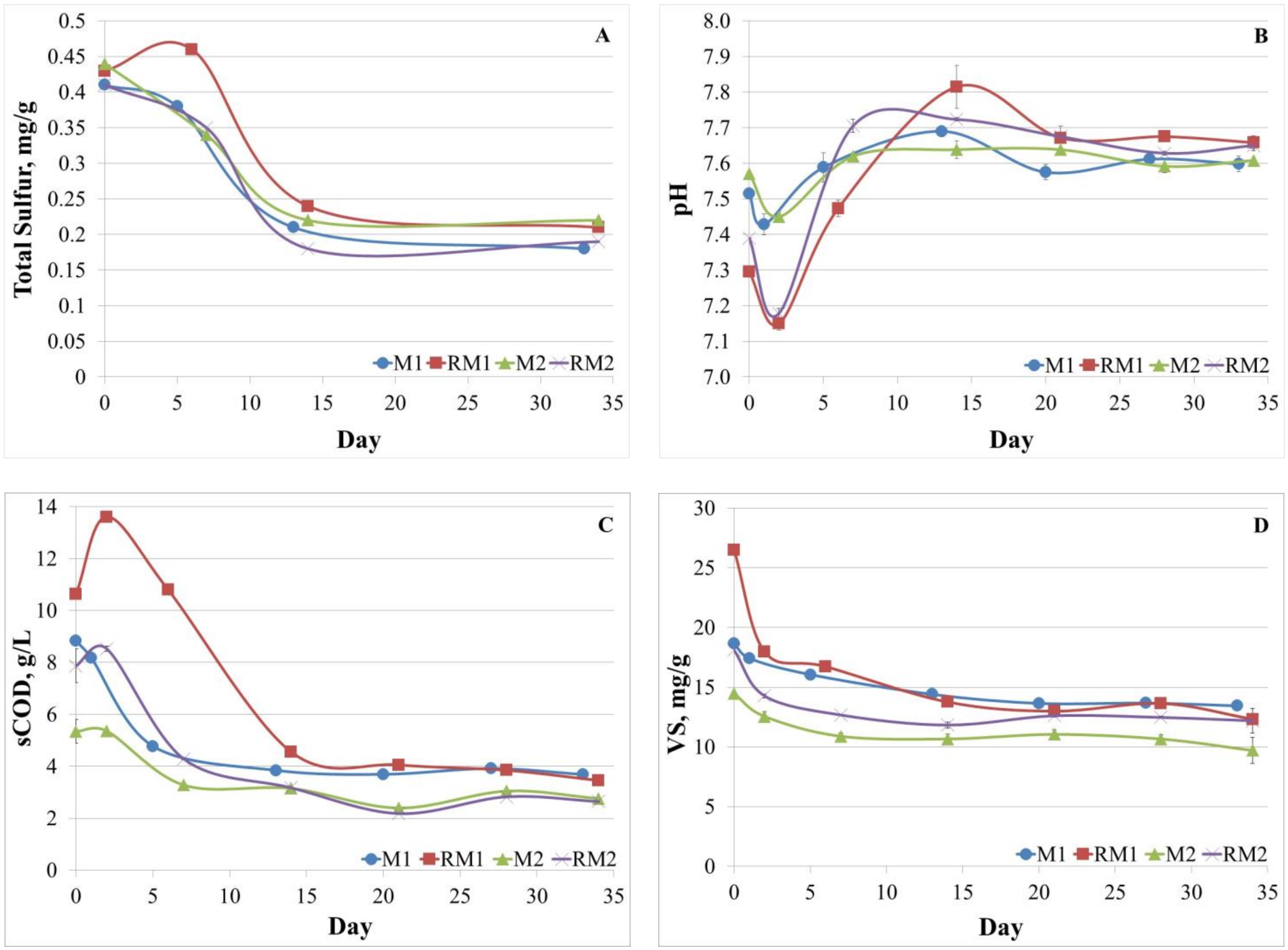

Figure 4.2 Characteristics of the manure-only and radish + manure digesters during 33 days of incubation. **(A)** mg S/g feedstock added, **(B)** pH, **(C)** g sCOD/L feedstock added, and **(D)** mg VS/g feedstock added.

Sulfur concentrations in the digesters followed a similar pattern during both trials. In the first trial, the total sulfur content of RM1 remained constant at ~ 0.45 mg S/g feedstock during the first six days of incubation, decreased by nearly 50% to 0.24 mg/g between Days 6 and 14, and then remained relatively constant for the remainder of the incubation period. For M1, RM2, and M2, sulfur concentrations decreased slightly during the first six days of digestion, decreased more steeply between Days 6 and 14, and remained stable thereafter. By Day 14, all digesters had similar total sulfur concentrations (0.18 – 0.22 mg S/g feedstock).

Although initial total sulfur concentrations were similar between M1 and RM1 and between M2 and RM2, the radish + manure digesters produced significantly more H_2S than the respective manure-only digesters (p-values = 0.009 and < 0.0001 for Trial 1 and 2, respectively). This suggests that the radish substrate was more readily degradable than the manure, allowing for the sulfur substrate in the radish + manure digesters to be rapidly converted to H_2S. RM1 produced 2.21 L H_2S/kg VS in comparison to M1 (1.71 L H_2S/kg VS), while RM2 produced 1.68 L H_2S/kg VS compared to M2 (0.91 L H_2S/kg VS). With more H_2S being produced in the radish + manure digesters, CH_4 production was suppressed initially. A similar result was shown during the digestion of apple waste. In those experiments, elevated H_2S concentrations and slow increases in CH_4 production during the initial incubation illustrated that SRB activity was greater than methanogenic activity (Kafle and Kim, 2013). In that study, the authors predicted that acidification of the apple waste may have contributed to the low methanogenic activity during the initial incubation. However, in the present study, no significant acidification of the forage radish was observed as pH remained relatively constant over the entire incubation period

(Figure 4.2). Our results suggest that it was the sulfur content of the radish and its rapid degradation that lead to significantly higher H_2S production which suppressed CH_4 production initially rather than pH causing CH_4 suppression. Similarly Kafle et al. (2014) demonstrated that the AD of another Brassica crop, Chinese cabbage waste, produced biogas with less than 50% CH_4 content during the initial days (<21 days) of incubation, with H_2S concentrations exceeding 5000 ppm. However after 21 days, the H_2S concentration gradually declined and the CH_4 concentration increased to ~80%.

The initial lag in CH_4 production observed in the radish + manure digesters could be due to a longer period of acclimatization required for digesting the radish cover crop or methanogenic inhibition by SRB. Brassica cover crops (i.e. camelina, radish fodder, mustard) have high levels of sulfur-containing glucosinolates. 30.3% of the total sulfur content in radish cover crops are glucosinolates (Peu et al., 2013). Although glucosinolate content is highly dependent on species, variety, and cultivar, radish was found to contain the highest concentration of glucosinolates (64 – 332 mg/100g) relative to other crucifers such as cabbage, turnip, and kale (20 – 151 mg/100g) (Ciska et al., 2000). Future studies should include a full sulfur analyses in the liquid and gas phase to further elucidate the lag phase observed. Additionally, the lag phase seen during batch digestion may not be present during continuous operation.

Although H_2S can have a corrosive effect on digestion systems, this research suggests that at peak CH_4 production co-digestion with the forage radish crop generates biogas that would not require any additional desulfurization beyond the standard practice of scrubbing the biogas from digestion of dairy manure prior to use in a CHP system. All digesters in the study had H_2S in the biogas above 0.05% (target concentration for CHP engines). On average, RM1 and RM2 had a H_2S concentration of approximately 0.20% (2000 ppm) during peak CH_4 production, with the manure-only digesters being slightly higher at 0.40% (4000 ppm) and 0.34% (3400 ppm) for M1 and M2, respectively (Figure 4.1). During peak CH_4 production, the radish co-substrate lowered the H_2S concentration of the biogas below the manure-only digesters by Days 9 and 7 for Trial 1 and Trial 2, respectively, although cumulatively the radish + manure digesters produced a larger quantity of H_2S when normalized by VS addition.

Overall, CH_4 production was not severely suppressed by radish addition during this batch process and was able to reach high concentrations of CH_4 after the initial lag phase. Similarly, Peu et al. (2013) demonstrated that the digestion of Brassica crops was not severely inhibited by glucosinolate content, but H_2S production in the biogas was not directly measured in their study. Peu et al. (2012) predicted that based on total sulfur content and biogas production that digestion of radish cover crops would produce biogas containing H_2S concentration $\leq$ 1.0% (v:v). Although the radish species and variety were not specified and the whole plant was used as substrate, the predicted value is similar to our findings in which only the radish above-ground biomass was used for co-digestion in order to maintain the soil nutrient benefits of the cover crop and reduce harvesting labor.

4.4.3 pH, nutrients, and organic matter transformations

Use of forage radish as a co-digestion substrate at an ISR of 53:47 required no pH adjustments during the incubation period. All digesters remained within the optimal pH range (6.5 – 8.0) for mesophilic AD (Figure 4.2; Table 4.4). Although the forage radish had an initial pH of ~4.5, adequate buffering capacity was maintained in this study for a circum-neutral digestion environment.

Table 4.4 Characteristics of the manure-only and radish + manure mixtures before and after digestion.

	M1		**RM1**		**M2**		**RM2**	
	Initial	*Final*	*Initial*	*Final*	*Initial*	*Final*	*Initial*	*Final*
pH	7.5 (0.02)	7.6 (0.02)	7.3 (0.01)	7.7 (0.02)	7.6 (0.00)	7.6 (0.01)	7.4 (0.00)	7.7 (0.02)
sCOD *(g/L)*	8.83 (0.05)	3.69 (0.18)	10.6 (0.1)	3.46 (0.02)	5.34 (0.46)	2.76 (0.03)	7.87 (0.64)	2.64 (0.01)
TS *(mg/g)*	27.6 (0.1)	22.3 (0.4)	36.9 (0.2)	22.5 (0.1)	21.4 (0.1)	16.7 (1.5)	26.1 (0.2)	21.1 (1.5)
VS *(mg/g)*	18.7 (0.1)	13.4 (0.3)	26.5 (0.2)	12.3 (0.1)	14.5 (0.04)	9.7 (1.1)	18.1 (0.2)	12.2 (1.0)
Sulfur *(mg/g)*	0.41	0.18	0.43	0.21	0.44	0.22	0.41	0.19

The addition of forage radish as a co-substrate also did not diminish the fertilizer value of the digester effluents (Table 4.5). TKN values for the manure-only and radish + manure digester effluents were not statistically different at 1695 ± 100 and 1764 ± 118 mg/L respectively, while TKP values were also not statistically different at 269 ± 16 and 278 ± 20 mg/L, respectively. This resulted in the digester effluents having an average N:P ratio of 6.3:1, which is close to the N:P ratio required by corn grain (6:1) (Paschold et al., 2008). The effluent of both digestion systems with and without radish would be an advantageous liquid fertilizer for corn-silage based dairy farmers.

The sCOD values increased in RM1 and RM2 during the first two days of digestion, likely due to the initial hydrolysis of the forage radish into simple soluble compounds (Figure 4.2). After the initial spike, sCOD for RM1 decreased by 10.2 g/L during the 33-day incubation period, with 90% of the reduction occurring during the first 14 days. In RM2, sCOD values were reduced by 5.9 g/L. There was less sCOD total destruction in the M1 and M2 digesters (5.1 and 2.6 g/L, respectively) due to the lower initial values. However by the end of the incubation period, the sCOD concentrations were similar between RM1 and M1, as well as between RM2 and M2, illustrating the ability of the radish + manure digesters to utilize the additional sCOD input from the radish substrate. VS reductions were similar, with 14.2 and 5.9 mg of VS removed per gram of feedstock addition for RM1 and RM2, respectively, and 5.2 and 4.8 mg/g for M1 and M2, respectively (Figure 4.2).

Table 4.5 Nitrogen and phosphorus characteristics of the digester effluents after 33 days of incubation.

	M1	RM1	M2	RM2
TKN *(mg/L)*	1750 (220)	1800 (260)	1640 (40)	1720 (40)
TKP *(mg/L)*	278 (32)	274 (44)	259 (10)	283 (9)
N:P	6.3:1	6.6:1	6.3:1	6.1:1

4.4.4 Farm-scale analysis

Harvesting the above-ground biomass of forage radish using a rotary mower and forage chopper, yielded 7,340 ± 1,050 kg/acre (fresh weight) of radish shoots and above-ground roots. Based on the pilot-scale CH_4 production results for RM2 (13% radish and 87% liquid dairy manure (ww basis)) and an ISR of 53% inoculum, a 200-dairy cow farm would require a 32 m^3 batch digestion system, assuming a 15% biogas headspace (Table 4.6). Operating the batch digester with a 30-day retention time would require the following substrates: 1630 kg (ww) of radish (corresponding to the yield from 1/4 acre) and 10,890 kg (ww) of manure, which is equivalent to the daily manure production from 200 dairy cows, assuming a daily manure production rate of 120 lbs/dairy cow (ASAE, 2003). The calculated CH_4 production from this batch system would be 105 m^3/month, which corresponds to a monthly energy yield of $3.65 * 10^6$ BTU or 1070 kWh, and is 77% greater than digesting 100% dairy manure.

Due to the low pH of forage radish (4.5) and volatile fatty acid production during digestion, adequate buffering capacity is required for optimal CH_4 production when digesting radish. A high volume of inoculum and/or manure would likely be required, especially if operating in batch mode. Other studies have shown that high inoculum/manure loads enhanced CH_4 production when digesting Brassica crops. Peu et al. (2013) utilized pig slurry for radish fodder (whole plant) co-digestion (75:25, ww), whereas Carvalho et al. (2011) used previously digested wastewater treatment plant sludge as inoculum for oilseed radish (whole plant silage) digestion (91:9, ww), with both being higher than the ISR used in this study (53:47).

Extrapolating from the batch digestion studies to a continuously operated digestion system would require a 460 m^3 digester for a 30-day retention time with manure loaded from 200 dairy cows and 1630 kg of radish daily, corresponding to seven acres of harvested radish per month. This assumes a one-time loading of 53% inoculum at start-up (Table 4.6). The CH_4 production from this system was calculated to be 3150 m^3/month, assuming the CH_4 production rate of the batch system. The CH_4 production value assumes that the pH would remain the same in a continuous system when inoculum was only added at start-up. However, this may not occur. If fluctuations in pH occurred, the radish content could be lowered for continuous operation.

For field-scale operation, the entire daily manure load of the farm could be treated and the amount of radish harvested and added to the digester would be based on the daily manure production rate. For a new digester operation, the additional digester capacity required for radish co-digestion could be taken into account at the design phase. For an existing system, the operator has several options including decreasing in-vessel gas storage space or retention time, or storing the radish and adding a smaller percentage of radish daily in order to accommodate the existing digester size.

Table 4.6 Energy and revenue per month for a 200-dairy cow farm operating a complete mix digestion system.

	Batch Digester	**Continuous Digester**
Retention time (days)	30	30
Feedstocks		
Forage radish[a] (kg_{ww})	1,630	48,800
Dairy manure[b] (kg_{ww})	10,890	326,590
Inoculum (kg_{ww})	14,110	14,110
Total (kg_{ww})	26,620	389,500
Digester size[c] (m^3)	32	460
CH_4 production[d] (m^3)	105	3,150
Energy yield (BTU)	3.65E+06	1.09E+08
Energy yield (kJ)	3.85E+06	1.15E+08
Energy yield (kWh)	1,070	32,050
Cover crop cost-share[e] (US$/acre)	80	80
Radish Substrate Value (US$)	20	560
Natural gas[f] (US$/$m^3$)	0.43	0.43
Electricity[g] (US$/kWh)	0.10	0.10
Co-digestion Revenue		
Gas (US$)	45	1,350
Electricity (US$)	105	3,125
Total Revenue		
Gas (US$)	65	1,910
Electricity (US$)	125	3,685

[a] Based on 7,340 kg of above-ground radish biomass/acre.
[b] Assumes a daily manure production rate of 120 lbs/dairy cow.
[c] Assumes a 15% volumetric headspace.
[d] Based on 8.38 L CH_4/kg substrate.
[e] Assumes a base payment, plus add-on incentives: cover crop planted by October 1 on a farm located in a targeted watershed on fields that previously grew corn and were fertilized in Spring with manure.
[f] Based on the average price of natural gas (2013 US$).
[g] Based on average price of electricity (2013 US$).

On a practical standpoint, our experience showed that utilizing a reduced radish loading resulted in more favorable digester operation and maintenance conditions. Reducing the radish content minimized clogging in the digester pumping system and allowed for the mechanical mixers to adequately agitate substrates without increased strain, thus reducing stratification of substrates in the digester. The smaller radish addition could also be more advantageous to the dairy farmer since less land would need to be harvested for the digestion co-substrate, resulting in reduced fuel costs associated with harvesting and transportation and less labor and energy would be required for harvesting and mechanically reducing the particle size of the radish.

A possible scenario for a corn-silage based dairy farmer utilizing a continuous complete mix digester would consist of the following. The forage radish cover crop would be planted in August immediately after corn harvest. Starting November, the forage radish cover crop would be harvested for co-digestion substrate weekly. Due to the consistency of the above-ground radish biomass after mowing and forage chopping, it is more ideal to harvest the radish in small batches. The colder temperatures should allow for the harvested radish to be stored outdoors. The ambient temperature will dictate if weekly radish harvesting can continue through December and how many additional acres could be harvested for continual loading in December and January. Typically in December or January, the forage radish cover crop will winter-kill after several consecutive nights with temperatures below - 6°C. The forage radish cover crop that will be utilized as co-substrate should be harvested prior to any predicted hard frost.

Through cost-share programs, farmers can receive grants for planting cover crops. Depending upon the farming practice, the average base payment is $45/acre. Using highly valued planting practices, such as planting in fields that previously grew corn or were fertilized with manure in the springtime can increase the payment to $80/acre (MDA, 2014). Utilizing the above-ground radish biomass as co-substrate could also increase farm revenue through biogas and electrical generation. Based on CH_4 production values from the batch and continuous digestion systems on a 200-dairy cow farm and the 2013 average price of natural gas (0.43 US$/$m^3$) and electricity (0.10 US$/kWh) (EIA, 2014), co-digesting with radish could provide additional revenue up to $45 and $1350/month, respectively, for natural gas and $105 and $3125/month, respectively, for electricity.

4.5 Conclusions

Increased renewable energy production can be realized through co-digestion with forage radish cover crops in dairy manure digesters. Although cumulative H_2S production in the radish + manure digesters was significantly higher than the respective manure-only digester, H_2S production in the radish + manure digesters rapidly declined, resulting in higher levels of CH_4 ($\geq$ 67%) and lower levels of H_2S (0.20%) in the biogas compared to the manure-only digesters during peak biogas production. For a 200-dairy cow farm, a continuous complete mix digester (30-day retention time) would require seven acres of radish and has the potential to produce 3150 m^3 CH_4/month through co-digestion.

4.6 Acknowledgements

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Appendix E contains supplementary tables for Chapter 4 detailing cumulative biogas, CH_4, and H_2S production values for the triplicate complete mix batch digesters.

Chapter 5: Forage Radish Cover Crops: Cultivation, Harvest, and Energy Yield

5.1 Cultivation

Forage radish is listed among the cover crops that the Maryland Agricultural Water Quality Cost-Share (MACS) Program provides farmers incentive payments for if planted by mid-September (Figure 5.1). According to Maryland's 2014-2015 cover crop program, the traditional cover crop (i.e. crop not harvested; crop grazed or chopped for on-farm livestock feed) base payment for planting radish is $45/acre. Add-on incentives are also available up to $90/acre for using highly valued farming practices. Farmers can also participate in the harvested cover crop program, which receives $25/acre (MDA, 2015a; 2015b).

The planting period in Maryland for forage radish is August 15-Septemeber 15. A minimum of five acres must be planted to participate in the cost-share. The seeding rate for forage radish required by MACS is 10 lbs/acre, although the rate should be increased by 25% for aerial application (MDA, 2015a; 2015b). However, if precisely planted or drilled, 6 to 10 lbs/acre is generally recommended (Gruver et al., 2014).

Although vegetable radish seeds can be cost-prohibitively expensive, there are several other varieties (i.e. GroundHog Radish; NitroRadish; Tillage Radish) of radish commonly marketed for cover cropping (Gruver et al., 2014). Survey of current seed prices in 50 lb bag lots revealed that radish seeds ranged from $2.40 to $3.45 per pound (Forage Seeds, 2015; Best Forage, 2015; Hancock Seed, 2015). If five acres of land were sown at a seeding rate of 10 lbs/acre and $3/lb for seeds, 50 lbs of seeds would be required costing $150 dollars ($30/acre) in total.

5.2 Harvesting

Forage radish (*Raphanus sativus* var. *longipinnatus*) cover crops were sown in August in Beltsville, MD after corn-silage harvesting. The radish was grown on residual nutrients, with no additional fertilizer or manure applied other than that which was used for the preceding corn silage crop. In early December, before winter-kill, radish biomass yields were determined. To quantify the whole radish biomass yield, shovels were used to extract the shoots and below-ground fleshy roots from 1m^2 quadrants in the field (Figure 5.2). Harvesting the whole radish resulted in a fresh weight yield of 24,807 ± 1,274 kg/acre (Table 5.1). A rotary mower was used to separate the above-ground radish biomass (shoots plus a small portion of the root that extended above the soil surface) from the below ground root (Figures 5.3 & 5.4). Mowing the radish resulted in an above-ground biomass yield of 11,139 ± 1,144 kg/acre. This harvesting method left the below ground root intact in the soil. It is estimated that at least 55% of the whole plant (below-ground root) remained in the field for decomposition.

Table 5.1 Biomass yields (± standard error) of forage radish cover crops.

	Fresh Biomass yield, kg/acre
Whole radish	24,807 (1,274)
Mowed radish (above-ground crop)	11,139 (1,144)
Mowed and chopped radish (above-ground crop)	7,344 (525)

In order to harvest the radish for use as an anaerobic digestion substrate, the radish was mowed and windrowed, followed by harvesting with a forage chopper. The chopped material was blown into an adjacent wagon for collection and weighed (Figures 5.5 & 5.6). This resulted in a biomass wet weight yield of 7,344 ± 525 kg/acre. Observation of the field after harvesting revealed that some of the above-ground biomass material was not collected as the forage chopper passed over the mowed windrow. Based on 'mowed' and 'mowed and chopped' biomass yields, it is estimated that over 30% of the above-ground material was left on the field as ground cover. Considering that only the above-ground radish biomass was forage chopped and utilized on-farm as anaerobic digestion substrate, it is not entirely clear whether MACS would classify the radish as a traditional or harvested cover crop. However, harvesting a portion of the forage radish cover crop would actually promote the environmental goals of the MACS program by removing nitrogen and phosphorus from the field.

Logistics for harvesting the forage radish could be improved to allow for more of the above-ground biomass to be collected per square meter as well as controlling the significant volume of liquid that is released during harvesting. High performance farming machinery, such as a sealed self-loading/unloading forage wagon, may allow for a cleaner gathering of the crop from the field and conservation of the liquid portion which may have a high energy content. Utilizing this type of wagon could also result in faster radish harvesting and reducing the amount of farm labor and machinery required.

For anaerobic digestion, fresh radish material was only utilized as substrate and therefore is only available until winter-kill (up to 4 months October-January). To increase availability of the radish year-round, storage options such as ensiling could be explored. However, the moisture content of the radish crop is high (90%), and thus might require a drier co-silage substrate to produce optimal silage. Additionally, the CH_4 yield of the silage should be determined before utilizing the material as a feedstock.

5.3 Processing

To determine the most suitable procedure for loading the radish into the complete mix digesters, several digester loading trials were conducted. Initially, the forage chopped radish was homogenized with dairy manure and loaded into the digester using a centrifugal pump. However due to the long fibrous radish strands (up to 10 cm), residual corn stalks, and other field debris, the pump's impeller and volute would clog resulting in the pump shutting off. To avoid damaging the pump and minimizing clogging, a Hobart industrial vertical cutter was used to further reduce the particle size of the forage chopped radish to less than 3 cm (Figure 5.7).

Logistics for processing the radish for anaerobic digestion could also be improved. To minimize energy consumption and labor, it would be ideal to have a harvesting method in the field that could simultaneously reduce the particle size of the radish during forage chopping into a form that was more suitable for conveyance into the digester. For farm-scale operation, it is recommended for all plumbing to be greater than 2 inches and to utilize a grinder pump to minimize clogging. Additionally, it is not recommended to utilize radish as a co-digestion substrate in dairy manure-based gravity fed plug flow digesters due to the radish separating from the manure during conveyance and clogging the plumbing.

5.4 Energy Yield

Utilizing forage radish cover crops as an anaerobic digestion substrate produced energy without land use competition. Biochemical methane potential (BMP) experiments conducted in the laboratory revealed that digestion of the above-ground radish biomass at an inoculum to substrate ratio of 91:9 (wet weight) had a specific CH_4 yield of 337 m^3/t

VS. Considering the biomass yield of 20 t/ha (7,344 kg/acre) and the specific CH_4 yield, the calculated CH_4 yield of the above-ground radish biomass was 532 m^3/ha. Since the specific CH_4 yield for radish was determined in small BMP vessels (300 mL), a more accurate reflection of CH_4 potential should be determined using larger-scale digesters for real-world radish only applications.

In comparison to other energy crops, the calculated CH_4 yield per hectare for radish was low (Table 5.2). It should be noted that common energy crops such as maize and wheat have over 4 times more VS content than forage radish, while red clover and oilseed rape have 2 to 3 times higher VS, respectively (Cropgen, 2008). Additionally, the Brassica crops, oilseed rape and forage radish had lower CH_4 yields per hectare. With maize having higher biomass yields and VS content, it was expected for the CH_4 yields per hectare to be higher than forage radish as shown. Additionally, although the biomass yields for oilseed rape were lower in comparison to radish, with 3 times more VS content in the oilseed rape, it is reasonable for the oilseed rape to also have higher CH_4 production as shown. Although, the calculated CH_4 yield per hectare was low for forage radish, its use as an anaerobic digestion substrate is still promising considering its readily available, does not compete with food production, provides multiple soil and environmental benefits, receives crop-share payments, and optimizes CH_4 production of a manure digester. To determine the sustainability of digesting forage radish and to access the energy return on energy invested, a cost benefit and energy analysis should be conducted.

Table 5.2 Biomass and energy yields of crops.

	Biomass yield, t/ha	Specific methane yield, m^3/t VS	Calculated methane yield, m^3/ha
Maize (whole crop)*	9 - 30	397 - 618	3,573 - 18,540
Wheat (grain)*	3.6 - 11.75	384 - 426	1,382 - 5,005
Red clover*	5 - 19	300 - 350	1,500 - 6,650
Oilseed rape*	2.5 -7.8	240 - 340	600 - 2,652
Radish (above-ground crop)	20	337	532

*Values obtained from Braun et al., 2009

Figure 5.1 A stand of forage radish cover crops in Beltsville, MD.

Figure 5.2 Extracting radish (whole crop) from $1m^2$ quadrants to determine biomass yield.

Figure 5.3 The above-ground radish biomass.

Figure 5.4 A rotary mower during radish harvesting.

Figure 5.5 A forage chopper and adjacent collection wagon during radish harvesting.

Figure 5.6 Forage chopped radish in collection wagon.

Figure 5.7 Foraged chopped radish in Hobart industrial vertical cutter.

Chapter 6: Conclusions

6.1 Results Summary

Forage radish cover crops are a suitable co-substrate for increasing methane (CH_4) production of a dairy manure-based digester. The cover crop had 1.5 fold higher CH_4 potential than dairy manure on a volatile solids basis. A farmer could harvest the above-ground biomass for anaerobic digestion co-substrate from October until December (winter-kill), as harvest date of the radish cover crop did not influence CH_4 production. Increasing the radish content of the co-digestion mixture and the inoculum to substrate ratio, significantly increased CH_4 production. Due to the low pH and rapid biodegradability of radish, adequate buffering capacity with the use of inoculum and/or dairy manure was required to prevent volatile fatty acid accumulation, which severely suppressed CH_4 production.

Initial H_2S production increased as the radish content of the co-digestion mixture increased, but the sulfur-containing compounds were rapidly utilized. Overall, the sulfur content of the radish did not suppress CH_4 production after an initial lag phase. Pilot-scale experiments revealed that co-digesting with radish increased CH_4 production by 39% relative to manure-only digestion. Additionally, the results suggest that at peak CH_4 production co-digestion with the forage radish crop generates biogas that would not require any additional desulfurization beyond the standard practice of scrubbing the biogas from digestion of dairy manure prior to use in a combined heat and power system. Extrapolated to a farm-scale (200 cows) continuous mixed digester, co-digesting with a 13% radish mixture could generate 3150 m^3 CH_4/month, providing a farmer additional revenue up to $3125/month in electricity sales.

Through cost-share programs, farmers can receive incentive payments for planting forage radish cover crops. Utilizing the radish as anaerobic digestion substrate produced energy without land use competition, thus expanding the use of the cover crop beyond improving topsoil fertility and alleviating soil compaction. Harvesting of the radish with a rotary mower and forage chopper yielded 20 t/ha of above-ground biomass, which has a calculated energy yield of 532 m^3 CH_4/ha.

6.2 Broader Impacts

It is expected that this co-digestion research will provide dairy farmers the tools needed to enhance CH_4 production in digesters during the fall and winter months by utilizing optimal co-digestion and inoculum to substrate ratios. Utilizing forage radish cover crops will provide multiple benefits to the farmer through soil nutrient management, water quality improvement, as well as the enhanced production of renewable energy. Through anaerobic digestion, not only is it expected to reduce CH_4 emissions associated with the handling of dairy manures, but it is also hopeful to provide farmers with a potential source of revenue (biogas), odor control, and the creation of an optimized fertilizer.

The funding provided by the Northeast Sun Grant and the University of Maryland has allowed many opportunities to educate diverse audiences locally, nationally, and internationally about advances in anaerobic digestion. This research has been presented to industry and the scientific community in poster and oral presentations at venues such as the American Ecological Engineering Society Conference, the American Society of Agricultural and Biological Engineering Annual International Meeting, and the Science for Biomass Feedstock Production and Utilization National Conference. Workshop presentations include the Clark School Engineering Sustainability Workshop, Bioscience Research and Technology Review Days, and the Northeast Sun Grant Anaerobic Digestion Workshops. A peer-reviewed journal article on this research has also been published in *Bioresource Technology's* Special Issue on Thermo-chemical Conversion of Biomass.

6.3 Future Work

Before farm-scale application, it is recommended to determine how inclusion of radish cover crops influence CH_4 and H_2S production utilizing a pilot-scale continuous digestion system. It should be determined whether optimized CH_4 production is maintained when the inoculum is added only during digester start-up and alkalinity monitoring should be simultaneously conducted. A cost benefit and energy analyses would also determine the sustainability of utilizing this co-digestion practice on farm. Additionally, it is suggested to improve radish harvesting and processing logistics which are discussed further in Chapter 5.

Appendices

Appendix A: Pilot-Scale Complete Mix Anaerobic Digester Design

Six pilot-scale complete mix anaerobic digesters were designed and constructed at the USDA's Beltsville Agricultural Research Center (BARC). The digesters were constructed from 850 L conical high-density polyethylene tanks (Ace Roto-Mold; Hospers, Iowa USA). Additional valves, ports, and pipes were primarily constructed from EPDM rubber, PVC piping, and stainless steel. The digesters were equipped with silicone adhesive rubber heating blankets to maintain mesophilic (35^{o}C) digestion conditions. To encapsulate heat surrounding the digester, foil reflective frames were installed. The reflective frames were constructed from PVC, plastic fencing, and industrial strength aluminum foil. The temperature control panel was wired to continuously monitor and simultaneously control the digester's internal and external temperatures. Protective casings (temperature sensing wells) were also fabricated to house the internal temperature probes in order to prevent direct contact with liquid contents. Top-mounted digester stirrers were constructed using 1/15 hp Dayton right angle gear motors and custom-made steel stir rods (25 cm diameter beveled mixing blades). The digester stirrers were simultaneously controlled by an electronic timer. Each digester was leak tested prior to operation. Detected leaks were sealed with an additional layer of 100% silicon. The digester lids were air-tight when silicone sealed. However, it was noted that the digesters could not tolerate anything beyond slight pressurization, therefore biogas was continuously collected in biogas bags to avoid breaking the digester lid seals. The biogas collection system was designed using ~1000 L plastic bags (~7 mil thickness), which were equipped with custom-made gas collection ports. The biogas bags were heat sealed and tested for leakage with pressure gauges and a combustible gas leak detector. To prevent excess water vapor from entering the biogas bags, moisture traps and desiccant traps were designed and installed on each digester.

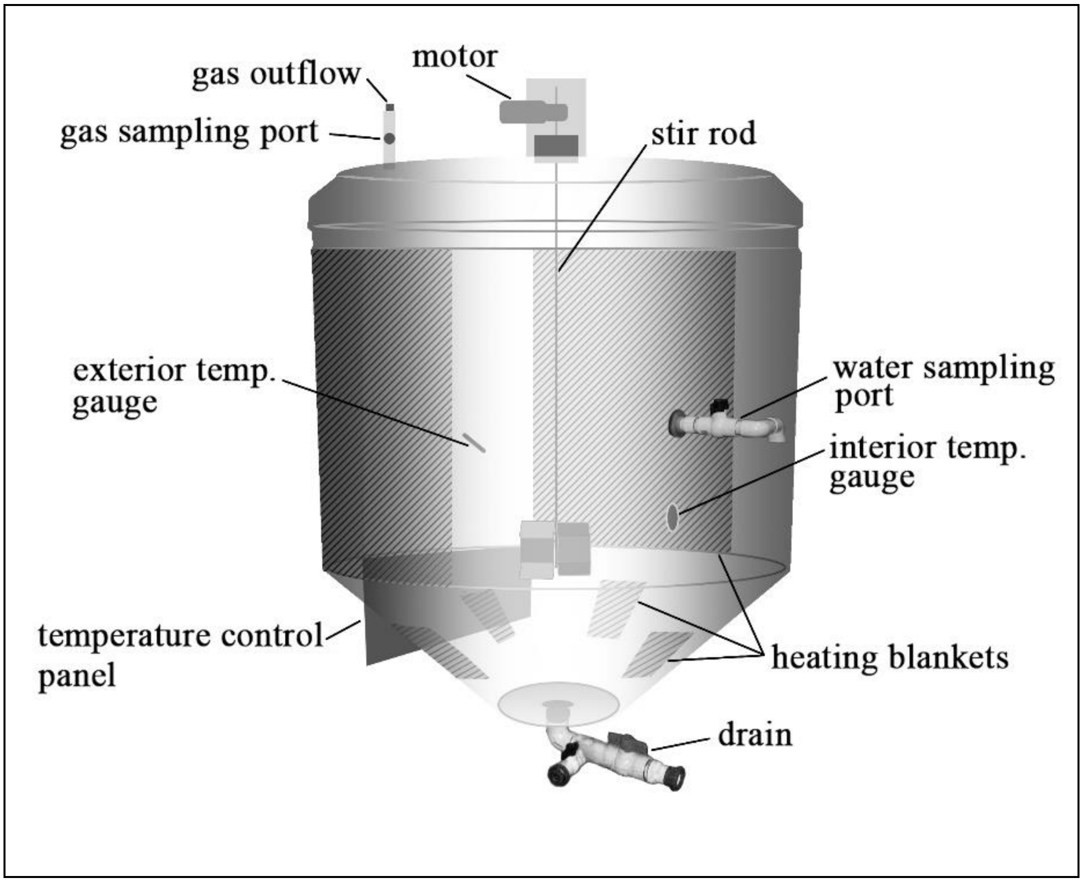

Figure A.1 Diagram of 850 L complete mix anaerobic digester.

Figure A.2 View of complete mix digesters under construction at USDA BARC.

(A)

(B)

Figure A.3 View of complete mix digester equipped with **(A)** silicone adhesive rubber heating blankets and **(B)** a foil reflective frame to encapsulate heat.

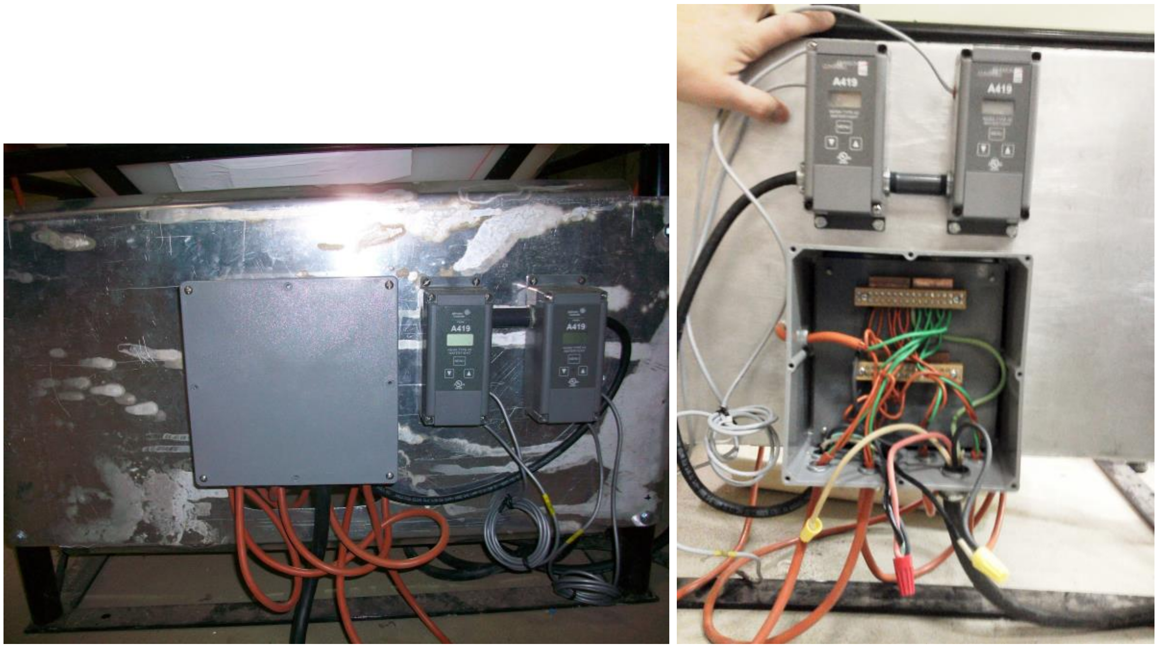

Figure A.4 View of temperature control panel.

Figure A.5 View of internal temperature probe enclosed in protective sensing well.

Figure A.6 View of top-mounted digester stirrers constructed using 1/15 hp Dayton right angle gear motors.

Figure A.7 View of beveled blade (25 cm diameter) steel stir rods.

Figure A.8 View of biogas bags equipped with gas collection ports.

(A)

(B)

Figure A.9 View of **(A)** moisture trap and **(B)** desiccant trap.

Figure A.10 Birdseye view of the complete mix digestion system.

Figure A.11 View of pilot-scale operational complete mix anaerobic digester.

Figure A.12 View of biogas bags in use.

Appendix B: Standard Operating Procedures for Complete Mix Digesters

B1. Biogas sampling

Materials

- Evacuated Tedlar gas bags (0.5 L)
- Graduated syringe with needle
- Small tube of silicone

Figure B.1 View of gas port with septum inserted.

Procedure

1. Evacuate Tedlar bags by vacuum pump.
2. Flush the graduated syringe several times with ambient air.
3. Biogas samples are obtained from the gas port.
4. Gently insert syringe needle into the gas port septum and withdraw 100 mL of biogas

5. Immediately expel biogas into a pre-evacuated Tedlar bag.
6. Apply a small amount of silicone to punctured septum.
7. Ensure that the syringe is flushed with ambient air in between sampling digesters.
8. The biogas samples are taken to the University of Maryland Water Quality Laboratory for analysis utilizing the gas chromatograph for CH_4 and H_2S content.

B2. Water sampling

Materials

- Buckets
- Sample bottles
- Ice packs
- Cooler

Procedure

1. Water samples are collected from the water sampling port.
2. Ensure that the stir rod is agitating throughout the sampling event.
3. Carefully open water sampling port and release about 100 mL of liquid and close valve. Discard liquid. Note that the liquid flow rate will be rapid.
4. Carefully open water sampling port valve again and discharge liquid into a clean bucket. Close valve. Divide the contents of the bucket into duplicate sample bottles.
5. Transport sample bottles on ice to the University of Maryland Water Quality Laboratory for analysis.

B3. Biogas metering

Materials

- Flathead screwdriver
- Foam insulation boards
- Small vacuum pumps rated at 2.5 m^3/h *(Caution: Be gentle. Handle with care! Pumps are made with glass canisters.)*
- Gas meters
- Logbook

Procedure

1. In warehouse breezeway, lay down 2 foam insulation boards per biogas bag.
2. Place small vacuum pump outdoors.
3. Plug in the small vacuum pump to an extension cord.
4. Connect the vacuum pump outflow tubing to the gas meter inflow. Ensure that the gas meter is always in an upright position.
5. Connect the gas meter output to tubing and place the end of the tubing outdoors as far away from the warehouse as possible.
6. Close gas ports on digester and biogas bag.
7. Carefully remove biogas tubing from bag gas port by using a flathead screwdriver to loosen hose clamp. Use a rotating motion to gently remove the tubing while one hand is gripping the ball valve. Immediately insert a septum into the biogas tubing end to avoid gas loss.
8. Holding the top two corners of the biogas bag, carefully remove bag from the hooks and sling.

9. Carefully carry biogas bag out to warehouse breezeway and gently place on the foam insulation boards. The biogas bag gas port should always face upward.
10. Connect the vacuum pump inflow tubing to the biogas bag. Record the date, time, bag number, meter and pump number, and starting reading on the gas meter in the logbook.
11. Open the gas port on the biogas bag.
12. Immediately turn on the vacuum pump.
13. Monitor the biogas bag while draining, ensuring to minimize creases and stopping just before a vacuumed suction occurs in the biogas bag.
14. Turn off the vacuum pump.
15. Close the biogas bag gas port.
16. Record ending number on the gas meter in the logbook.
17. Return drained biogas bag to frame and re-attach with hooks. Secure sling to ensure the gas port remains parallel to the floor.
18. Reconnect biogas tubing to biogas bag. Secure tubing with a hose clamp with screwdriver. Ensure that the clamp is not too tight on tubing to avoid damaging tubing.
19. Open the gas ports on the biogas bag and digester.
20. Repeat until all bags are drained.
21. After all bags are drained, turn small vacuum pumps on and flush ambient air through, while it is still connected to the gas meter for 3 minutes.
22. Disconnect and store biogas metering set-up.

Figure B.2 View of biogas metering set-up.

B4. Maintenance

Daily maintenance for complete mix digestion system

1. Open doors for venting of warehouse upon arrival.
2. Inspect each digester to ensure stir rod is attached to motor (as marked by a red line on top and bottom of connecting barrel).
3. Ensure that digester motors are running smoothly.
4. Using the timer, turn off the stir rods. Tighten all screws, reapply vacuum grease to grommet surrounding stir rod, and turn stir rods back on.
5. Inspect temperature control panel to ensure unit is properly functioning. The interior temperature should range between 33 – 35˚C. If interior temperature

reaches 35°C or exterior temperature reaches 45°C, the temperature controllers will shut off until digester system cools.

6. Inspect each biogas bag to ensure that they are properly secured to frame.
7. Ensure all digester gas ports and biogas bags collection ports are open.
8. Check biogas tubing for moisture build-up. Empty water from moisture traps if necessary.
9. Check desiccant trap for moisture control and replace desiccant if necessary.
10. Ensure no visible water leaks or biogas leaks using combustible gas leak detector. Thoroughly inspect digester lid, motor mount with rod, and gas port as well as all seams and biogas collection port on the biogas bags. Immediately address any detected leak.
11. Check biogas sampling septum for leaks and replace septum if necessary.

B5. Desiccant traps

Materials

- Flat head screwdriver
- Septums
- Desiccant

Procedure

1. Disconnect desiccant trap from digester with flat head screwdriver. Ensure to stopper digester biogas tubing with septums to avoid loss of gas.
2. On desiccant trap, remove 1- Fernco (end connected directly to plastic clear tube).
3. Pour exhausted desiccant from trap into plastic bag.

4. Immediately replace with dry desiccant. Ensure to tightly pack the desiccant into the trap. Resemble desiccant trap and connect to digester.
5. Repeat to change out all desiccant traps as necessary.
6. At the University of Maryland Water Quality Laboratory, place exhausted desiccant (~1 inch thick layer) into a metal tray.
7. Allow to dry at 120 ^{o}C for 8 hours in drying oven.
8. Stir desiccant occasionally and monitor to avoid burning desiccant.
9. When dry, immediately transfer desiccant and store in air-tight container.

B6. Digester loading procedure

Materials

- Forklift
- Hobart industrial vertical cutter
- Scales (truck scale and industrial scale)
- 300-gallon tanks on heavy duty forklift pallets
- 50-gallon drums
- Inoculum
- Solids-separated liquid dairy manure
- Forage chopped radish
- 1.5 hp centrifugal pump
- Drum pump
- 2" connecting hose with banjo fitting
- 1.5" connecting hose with female union

Radish preparation

Evenly place 5-gallons of thawed forage chopped radish into the Hobart industrial vertical cutter. Blend for 1 min on high speed and check consistency to ensure particle size is less than 3 cm. It is vital to retighten the Hobart cutter blades between each load of radish as the blades have a tendency to loosen. Use specialized wrench and key.

Procedure

1. Transfer inoculum from BARC's complete mix digester (540 m^3) into a 300-gallon tank. Ensure drain port is closed on tank. Note that transferring the inoculum via a digester sampling port is a slow process.
2. After using the Hobart vertical cutter, weigh radish on industrial scale located in warehouse (163F).
3. Obtain solids-separated dairy manure in 50-gallon drums.
4. Place an empty 300-gallon tank on the truck scale outside of 163 F to obtain weight. Record weight. Ensure drain port is closed on tank.
5. Using a forklift, transfer inoculum tank and drums of manure to the truck scale area. Additionally, transport the pre-weighed radish to the truck scale area.
6. Using a drum pump, transfer the desired amount of inoculum into the empty tank on the truck scale. Record weight.
7. Using a drum pump, transfer the desired amount of manure into the tank on the truck scale. Record weight.
8. Transfer the desired amount of pre-weighed radish into the tank on the truck scale. Record weight.

9. Forklift the tank on the truck scale into the warehouse. Ensure to place the tank so that it is accessible with the length of hosing available to load each digester.
10. Thoroughly homogenize mixture in tank with a 2x4 piece of lumber.
11. Connect a 2” hose to the tank drain port and the 1.5 hp centrifugal pump input.
12. Connect a 1.5” hose to the pilot-scale digester drain port (located on bottom of digester) and the 1.5 hp centrifugal pump output.
13. While transferring feedstocks from tank to digester, ensure to continuously stir mixture.
14. Open the ball valve on the digester drain. Check to make sure that the water sampling port is closed on the digester. Remove the digester septum, open the gas port, and take the biogas tubing off the gas port.
15. Open the ball valve on the tank drain.
16. Check for any leaks in the set-up.
17. Turn the centrifugal pump on.
18. Pump feedstock into the digester while monitoring volume level on digester. Remember to keep homogenizing mixture in tank.
19. Ensure that the digester liquid level is always above the heating blankets to prevent scorching the digester walls.
20. Turn off the pump and quickly close the ball valves on the digester and the tank.
21. Close the digester gas port. Attach biogas tubing with a hose clamp to the gas port. Insert septum and secure with a hose clamp.
22. Attach the other end of the biogas tubing to an empty biogas bag.

23. Record tank number, biogas bag number, treatment (including weights of feedstocks added), date, and time in the logbook.
24. Disconnect pump carefully as liquid will still be in the hosing. Allow hoses to completely drain. Between treatments, flush pump and hoses with water to cleanse.
25. Repeat this process 5 times, selecting the digesters for manure-only vs. radish + manure treatments in a random order.
26. Once all digesters have been loaded, turn on temperature control panels and stir rods. Open digester gas ports and biogas bag ports.
27. Clean out the pump and hosing with water and wash down area.

Figure B.3 View of digester loading set-up.

B7. Digester unloading procedure

Materials

- Flathead screwdriver
- 300-gallon tanks on heavy duty forklift pallets
- 1.5” connecting hose with female union
- 1.5 hp centrifugal pump

Procedure

1. Connect a 1.5” hose to the pilot-scale digester drain port and direct outtake into a 300-gallon empty tank.
2. Remove septum and biogas tubing from digester gas sampling port with a flathead screwdriver. Keep gas port open.
3. Slowly open digester drain.
4. Observe carefully to prevent digester lid from collapsing.
5. Once the liquid level is just below the water sampling port, open the water port.
6. Continue to drain digester into the tank.
7. To clean digester, fill a 300-gallon tank with water and use the centrifugal pump to flush water into the digester several times. Agitate water with digester stir rod.
8. Wash down area when complete

Appendix C: Supplementary Tables for Chapter 2

Table C.1 Average cumulative biogas and CH_4 production values for BMP1 for the triplicate bottles, with 100 g_{ww} of inoculum added to each BMP bottle, including inoculum controls.

	Biogas[a] *(mL)*	**CH_4**[a] *(mL)*	**CH_4**[b] *(mL)*	**g VS added** *(substrate)*	**mL CH_4/g VS**[b]	**g substrate added**	**mL CH_4/g substrate**[b]
Inoculum	166	36					
0% Radish	313	121	85	0.36	236	10	8.49
20% Radish	412	176	140	0.45	315	10	14.0
40% Radish	498	219	183	0.53	344	10	18.3
50% Radish	522	230	194	0.57	337	10	19.4
60% Radish	578	256	220	0.62	357	10	22.0
80% Radish	631	279	243	0.70	345	10	24.3
100% Radish	743	330	294	0.79	372	10	29.4

[a] Inoculum included.

[b] CH_4 production from the inoculum source subtracted from each bottle.

Table C.2 Average cumulative biogas and H_2S production values for BMP1 for the triplicate bottles, with 100 g_{ww} of inoculum added to each BMP bottle, including inoculum controls.

	Biogas[a] *(mL)*	**H_2S**[a] *(mL)*	**H_2S**[b] *(mL)*	**g VS added** *(substrate)*	**mL H_2S/g VS**[b]	**g substrate added**	**mL H_2S/g substrate**[b]
Inoculum	166	0.16					
0% Radish	313	0.42	0.26	0.36	0.73	10	0.03
20% Radish	412	0.58	0.42	0.45	0.94	10	0.04
40% Radish	498	0.91	0.75	0.53	1.40	10	0.07
50% Radish	522	1.10	0.94	0.57	1.64	10	0.09
60% Radish	578	1.17	1.01	0.62	1.63	10	0.10
80% Radish	631	1.45	1.29	0.70	1.84	10	0.13
100% Radish	743	1.92	1.76	0.79	2.23	10	0.18

[a] Inoculum included.

[b] H_2S production from the inoculum source subtracted from each bottle.

Table C.3 Average cumulative biogas and CH_4 production values for BMP2 for the triplicate bottles, with 125 g_{ww} of inoculum added to each BMP bottle, including inoculum controls.

	Biogas[a] *(mL)*	**CH_4**[a] *(mL)*	**CH_4**[b] *(mL)*	**g VS added** *(substrate)*	**mL CH_4/g VS**[b]	**g substrate added**	**mL CH_4/g substrate**[b]
Inoculum	143	18					
Early Harvest Radish	839	411	394	1.11	355	26	15.1
Late Harvest Radish	802	403	386	1.12	345	26	14.8

[a] Inoculum included.

[b] CH_4 production from the inoculum source subtracted from each bottle.

Appendix D: Supplementary Table for Chapter 3

Table D.1 Average cumulative CH_4 production values for the inoculum to substrate ratio (ISR) study for the triplicate bottles, with varying ratios of inoculum added to each BMP bottle, including inoculum controls*.

	mL CH_4/g VS[a]	**g VS added** *(substrate)*	**CH_4**[a] *(mL)*	**Inoculum** *(g_{ww})*	**Inoculum factor**[b]	**Calculated CH_4**[c] *(mL)*	**Actual CH_4**[c] *(mL)*
Inoculum			1.55	20			
Substrate: 100% Radish							
ISR 65	6.15	1.66	10.2	37.1	1.86	13.1	13.0
ISR 50	0.67	1.66	1.11	20.0	1.00	2.66	1.48
ISR 35	0.02	1.66	0.03	10.8	0.54	0.87	0.03
ISR 20	0	1.66	0	5.00	0.25	0.39	0
ISR 10	0	1.66	0	2.22	0.11	0.17	0
ISR 0	0	1.66	0	0	0	0	0
Substrate: 40% Radish/60% Manure							
ISR 65	284	1.09	308	37.1	1.86	311	312
ISR 50	239	1.09	259	20.0	1.00	261	262
ISR 35	2.46	1.09	2.67	10.8	0.54	3.50	2.96
ISR 20	0.86	1.09	0.93	5.00	0.25	1.32	1.07
ISR 10	0.48	1.09	0.52	2.22	0.11	0.69	0.67
ISR 0	0.03	1.09	0.03	0	0	0.03	0.03

[a] CH_4 production from the inoculum source subtracted from each bottle.
[b] Adjusts for quantity of inoculum in each bottle relative to inoculum controls.
[c] Inoculum included.
[*] Actual CH_4 values determined by subtracting out inoculum CH_4 production on a daily basis, while the calculated values were based on cumulative CH_4 production. If the CH_4 production from inoculum was higher than the substrate CH_4 production on a certain day, the substrate daily CH_4 value was given as zero.

Appendix E: Supplementary Tables for Chapter 4

Table E.1 Average cumulative biogas and CH_4 production values for the triplicate complete mix batch digesters.

	Biogas[a] *(L)*	**CH_4**[a] *(L)*	**kg VS added** *(total)*	**L CH_4/kg VS**[a]	**kg substrate added**	**L CH_4/kg substrate**[a]
M1	4093	2779	14.6	190	365	7.61
RM1	7879	4681	22.3	210	365	12.8
M2	2790	1732	11.6	149	365	4.74
RM2	5459	3060	14.7	208	365	8.38

[a] Inoculum included.

Table E.2 Average cumulative biogas and H_2S production values for the triplicate complete mix batch digesters.

	Biogas[a] *(L)*	**H_2S**[a] *(L)*	**kg VS added** *(total)*	**L H_2S/kg VS**[a]	**kg substrate added**	**L H_2S/kg substrate**[a]
M1	4093	25.0	14.6	1.71	365	0.07
RM1	7879	49.3	22.3	2.21	365	0.14
M2	2790	10.5	11.6	0.91	365	0.03
RM2	5459	24.6	14.7	1.68	365	0.07

[a] Inoculum included.

Appendix F: Letter for Inclusion of One's Own Previously Published Materials

DEPARTMENT OF
ENVIRONMENTAL SCIENCE
& TECHNOLOGY

1449 Animal Sci./Ag. Eng. Bldg.
College Park, Maryland 20742
301.405.1197 TEL 301.314.9023 Fax
www.enst.umd.edu

April 6, 2015

Charles Caramello
Dean of the Graduate School
University of Maryland
2123 Lee Building
College Park, Maryland 20742

Dear Dean Caramello,

This letter serves to inform you that Ashley J. Belle's dissertation entitled, "Coupling Anaerobic Digestion Technology and Forage Radish Cover Cropping to Optimize Methane Production of Dairy Manure-based Digestion," contains her own previously published work that was recommended to be included as part of the final dissertation. The work is included in Chapter 4 of this dissertation and was previously published in *Bioresource Technology's* Special Issue on Thermo-chemical Conversion of Biomass. The published material is research that was conducted and primarily written by Ashley J. Belle during her doctoral studies at the University of Maryland. Ashley is listed as the first author of this publication, with members of her dissertation committee as co-authors. The Dissertation Director, along with the dissertation examining committee and the Department of Environmental Science and Technology's Graduate Director, accept the inclusion of this previously published work in the dissertation as Ashley has made substantial contributions to the work.

Sincerely,

Stephanie Lansing, PhD
Dissertation Director

Martin Rabenhorst, PhD
ENST Graduate Director

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